

NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for ISOPRENE

**FINAL
MARCH 1999**

Prepared for

the December 2-3, 1998,
Meeting of the Report on Carcinogens Subcommittee
of the NTP Board of Scientific Counselors

Prepared by

Integrated Laboratory Systems
Post Office Box 13501
Research Triangle Park, North Carolina 27709
NIEHS Contract No. N01-ES-25346

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NTP Report on Carcinogens Listing for Isoprene

Carcinogenicity

Isoprene is *reasonably anticipated to be a human carcinogen* based on evidence of benign and malignant tumor formation at multiple organ sites in multiple species of experimental animals (Melnick et al., 1994; NTP, 1995; NTP, 1997 draft; Placke et al., 1996). Inhalation exposure of mice to isoprene vapors induced increased incidences of neoplasms of the lung, liver, Harderian gland, forestomach, hematopoietic system, and circulatory system. Inhalation exposure of rats to isoprene vapors induced increased incidences of neoplasms of the mammary gland, kidney, and testis.

No studies on the potential carcinogenicity of isoprene in humans were identified.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Isoprene is the 2-methyl analog of 1,3-butadiene, an industrial chemical that has been identified as an animal and human carcinogen. Isoprene and butadiene are metabolized to monoepoxide and diepoxide intermediates by liver microsomal cytochrome P450-dependent monooxygenases from several species, including humans. Detoxification of these intermediates may occur by hydrolysis catalyzed by epoxide hydrolase or conjugation with glutathione catalyzed by glutathione-S-transferase. The diepoxide intermediates of isoprene and butadiene are mutagenic in *Salmonella typhimurium* whereas the parent compounds are inactive (Gervasi et al., 1985). In mice, isoprene and 1,3-butadiene induced sister chromatid exchanges in bone marrow cells and increased the frequency of micronucleated erythrocytes in peripheral blood (Tice et al., 1987; cited by NTP, 1997 draft; Tice et al., 1988). Common sites of neoplasm induction by isoprene and butadiene include the mammary gland and testis in rats, and the liver, lung, Harderian gland, forestomach, and circulatory system in mice (NTP, 1997 draft). Lung and Harderian gland neoplasms induced by isoprene in mice had a high frequency of unique *K-ras* mutations (A to T transversions at codon 61) (Hong et al., 1997).

No data are available that would suggest that mechanisms thought to account for tumor induction by isoprene in experimental animals would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or

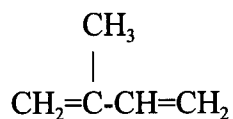
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 CHEMICAL PROPERTIES

Isoprene
[78-79-5]



1.1 Chemical Identification

Isoprene (C₅H₈, mol. wt. = 68.1) is also called:

Isopentadiene
β-Methylbivinyll
2-Methylbutadiene
2-Methyl-1,3-butadiene
2-Methyldivinyll
2-Methylerythrene

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Colorless	Budavari (1996)
Physical State	Liquid	Budavari (1996)
Melting Point, °C	-145.95	Budavari (1996)
Boiling Point, °C	34.067	Budavari (1996)
Density at 20 °C	0.6805	Budavari (1996)
Solubility:		
Water at 20 °C	Insoluble in water	
Organic Solvents	Miscible with ethanol or diethyl ether	Budavari (1996)
Partition Coefficients:		
Log octanol/water	2.42	Chem. Inspect. Test. Inst. (1992; cited by HSDB, 1997)
Vapor pressure at 25 °C	550 mm Hg	Zwolinski et al. (1971; cited by HSDB, 1997)
Conversion factor	1 ppm = 2.79 mg/m ³ at 25 °C and 760 mm Hg	Clayton and Clayton (1981-1982; cited by HSDB, 1997)

Isoprene is a highly flammable liquid. It has a flash point of -48 °C and is easily ignited by heat, sparks, or flames (U.S. DOT, 1996; cited by HSDB, 1997; Saltman, 1985). Vapors may form highly explosive mixtures with air and may polymerize explosively when heated. It is highly reactive, with reactions similar to those of 1,3-butadiene. In the absence of inhibitors, isoprene forms peroxides upon air exposure (Saltman, 1985).

Isoprene is one of the major photochemically reactive hydrocarbons emitted by numerous plant species (Bowling et al., 1998). The large quantities of non-methane hydrocarbons (NMHCs) emitted by vegetation, especially in tropical and subtropical regions, influence atmospheric processes. Isoprene and other highly reactive natural alkenes can serve as precursors to formation of photochemical oxidants that contribute to regional-scale air pollution (Hoffman et al., 1996). Isoprene, the monoterpenes, and other unsaturated hydrocarbons react with hydroxyl radicals (HO·) and tropospheric ozone (O₃) and may act as photochemical smog precursors. Since they compete directly with methane for hydroxyl radicals, they may indirectly affect the global warming trend (Loreto, 1997). Condensable oxidation products undergo gas-to-particle conversion, forming tropospheric organic particulates (Hoffman et al., 1996). However, Altschuller (1983) stated that the organic aerosols of the eastern United States formed from biogenic hydrocarbons do not contribute "a significant fraction" to urban and rural fine particulate aerosol concentrations.

The lifetime of atmospheric isoprene has been variously estimated to be 1.3 to 34.0 hours and 1 to 2 hours based on its rates of reactions with ozone and hydroxyl radicals. Some monoterpene species are more reactive with lifetimes less than 0.5 h (Altschuller, 1983; Guenther et al., 1995).

In sunlight, ultraviolet irradiation of isoprene, other biogenic NMHCs, and anthropogenic hydrocarbons in the presence of atmospheric nitrogen oxides (NO_x) gives numerous reaction products, including acetaldehyde, acetone, carbon dioxide, carbon monoxide, formaldehyde, formic acid, and peroxyacetyl nitrates (PAN). These products plus methacrolein and methyl vinyl ketone, which are apparently specific to isoprene, represent 30 to 73% of the carbon content of the reacted isoprene (Altschuller, 1983).

2.0 HUMAN EXPOSURE

2.1 Production

Isoprene is recovered from C₅ streams as a by-product of thermal cracking of naphtha or gas oil. The isoprene yield is about 2 to 5% of the ethylene yield (Saltman, 1985). U.S. demand for isoprene grew 6.5% annually from 1985 to 1992 (Chem. Mark. Rep., 1994; Chem. Week, 1994). In 1994, isoprene production in the United States was approximately 619 million pounds (281,000 Mg [metric tons]) (USITC, 1995), an increase of almost 29% over production in 1992 (USITC, 1994). Estimated isoprene production capacity for 8 facilities was 598 million pounds in 1996, based on estimates of isoprene content of product stream available from ethylene production via heavy liquids (SRI Int., 1997).

2.2 Use

About 95% of isoprene production is used to produce *cis*-1,4-polyisoprene; 2%, to produce butyl rubber (isobutene-isoprene copolymer); and 3%, to produce thermoplastic, elastomeric co-block (SIS) polymers (Saltman, 1985; Taalman, 1996).

2.3 Exposure

2.3.1 Endogenous

Isoprene is formed endogenously in humans; concentrations in blood range from 15 to 70 nmol/L (1.0 to 4.8 $\mu\text{g/L}$) (Cailleux et al., 1992; cited by NTP, 1997 draft). Humans produce isoprene endogenously at a rate of 0.15 $\mu\text{mol/kg/h}$ (Taalman, 1996) [about 17 mg/day for a 150-lb (70-kg) person]. [Endogenous production rates reported for rats and mice are 1.9 and 0.4 $\mu\text{mol/kg/h}$, respectively (Peter et al., 1987; cited by Taalman, 1996).] The availability and distribution of endogenous isoprene is partially controlled by the activated precursor isopentenyl pyrophosphate for the synthesis of biomolecules that contain isoprene units (NTP, 1997 draft).

Isoprene was the major hydrocarbon (up to 70%) in the air exhaled by all but one of 30 volunteers after they had breathed purified air for 10 min. The quantity exhaled per day per individual was reported as 2 to 4 mg and did not vary with age, sex, ethnicity, diet, life style, or fasting or nonfasting state. However, individuals showed day-to-day variations (Gelmont et al., 1981). The exhalation rate fell within the range of others reported—40 to 250 $\mu\text{g/h}$ or 0.96 to 6.0 mg/day for nonsmokers and 15 to 390 $\mu\text{g/h}$ or 0.36 to 9.36 mg/day for smokers (Clayton and Clayton, 1981-1982; cited by HSDB, 1997).

Breath concentrations in nonsmokers exhaling 1 to 6 mg isoprene/day can be estimated to be 0.05 to 0.4 mg/m³ based on the assumption that an adult breathes about 15 to 20 m³ air per day. Somewhat higher values of 10 to 30 nmol/L or 0.68 to 2.0 mg/m³ were reported by Cailleux and Allain (1989; cited by IARC, 1994). These estimates are comparable to more recent isoprene determinations in human breath. For example, baseline isoprene concentrations in alveolar breath of nonsmokers and smokers were 7.2 ± 0.5 and 6.2 ± 0.4 nmol/L (0.49 and 0.42 mg/m³), respectively, in the experiments of Euler et al. (1996) further described below. The isoprene concentration in the air breathed by the subjects was 0.18 ± 0.03 nmol/L (0.012 mg/m³). [In contrast to humans, Gelmont et al. (1981) reported that "significant amounts" of isoprene were not found in the breath of mice, guinea pigs, chickens, rabbit, and dog. Nursing rats and weanling rats eating sour cream and cottage cheese exhaled isoprene, but amounts decreased within 6 days of weaning.]

Analysis of the air of a parked car occupied by one person found that isoprene at 0.020 mg/m³ was the major volatile non-methane hydrocarbon (NMHC) when the car was not in a traffic-polluted place. The isoprene level was much lower in an unoccupied car, indicating that isoprene originates mainly from expired air (Bjorkvist et al., 1997).

As discussed below, ambient air concentrations of isoprene are generally less than about 10 ppb C or about 0.03 mg isoprene/m³, an order of magnitude less than the reported breath concentrations given above. Based on estimated human intake of 15 to 20 m³ air per day, ambient air would contribute less than 0.45 to 0.6 mg isoprene/day to daily exposure. The estimate of 17 mg/day given above for human endogenous production of isoprene is about 30- to 40-fold higher than the contribution from ambient air (calculations by ILS Inc.). Yet recently, Phillips et al. (1994), presented evidence that isoprene is not produced endogenously. The publication did not give the actual isoprene concentrations in breath of 12 volunteers and the air they breathed, but it showed graphically that the breath concentration was lower than that in the ambient air. They concluded that isoprene must come from an exogenous source and is then catabolized or excreted by a pathway other than the lungs (such as the kidneys or the liver).

2.3.2 Occupational

NIOSH collected data on potential exposure to isoprene in the National Occupational Hazard Survey (NOHS) from 1972 to 1974 (NIOSH, 1976) and in the National Occupational Exposure Survey (NOES) from 1981 to 1983 (NIOSH, 1990). These data are presented in Tables 2-2 and 2-3. The first survey (NIOSH, 1976) indicated that 58,000 employees in over 30 different industries were potentially exposed to isoprene. The more limited later survey of six industries showed that approximately 3,700 workers were potentially exposed to isoprene between 1981 and 1983 (NIOSH, 1990).

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TABLE 2-1. POTENTIAL INDUSTRIAL ISOPRENE EXPOSURES ESTIMATED IN THE 1972-1974 NATIONAL OCCUPATIONAL HAZARD SURVEY (NOHS) (NIOSH, 1976)

SIC CODE	DESCRIPTION	PLANTS	TOTAL EMPLOYEES	FEMALE EMPLOYEES
1511	GENERAL BUILDING CONTRACTORS	40	200	
1941	SIGHTING AND FIRE CONTROL EQUIPMENT	13	26	
2281	YARN MILLS, EXCEPT WOOL	14	14	
2329	MEN'S AND BOYS' CLOTHING, NEC	222	444	
2621	PAPER MILLS, EXCEPT BUILDING PAPER	6	1,051	
2651	FOLDING PAPERBOARD BOXES	94	5,280	
2751	COMMERCIAL PRINTING, EX LITHOGRAPHIC	113	2,262	
2818	INDUSTRIAL ORGANIC CHEMICALS, NEC	68	270	
2822	SYNTHETIC RUBBER	20	6,316	
2851	PAINTS AND ALLIED PRODUCTS	47	3,111	
2893	PRINTING INK	23	46	
2992	LUBRICATING OILS AND GREASES	18	234	
3021	RUBBER FOOTWEAR	8	8	
3069	FABRICATED RUBBER PRODUCTS, NEC	33	606	
3291	ABRASIVE PRODUCTS	31	778	
3411	METAL CANS	10	20	
3481	MISC. FABRICATED WIRE PRODUCTS	35	71	
3531	CONSTRUCTION MACHINERY	56	56	
3564	BLOWERS AND FANS	23	69	
3622	INDUSTRIAL CONTROLS	27	108	
3632	HOUSEHOLD REFRIGERATORS AND FREEZERS	24	353	
3651	RADIO AND TV RECEIVING SETS	38	943	
3662	RADIO AND TV COMMUNICATION EQUIPMENT	7	28	
3731	SHIP BUILDING AND REPAIRING	20	490	
3742	RAILROAD AND STREET CARS	20	39	
3821	MECHANICAL MEASURING DEVICES	14	1,571	
4131	INTERCITY BUS LINES	25	422	
4511	CERTIFICATED AIR TRANSPORTATION	24	24	
4832	RADIO BROADCASTING	40	356	
4924	NATURAL GAS DISTRIBUTION	499	2,118	
5092	PETROLEUM AND PETROLEUM PRODUCTS	164	14,596	
5311	DEPARTMENT STORES	57	342	
5321	MAIL ORDER HOUSES	22	22	
7929	ENTERTAINERS' & ENTERTAINMENT GROUPS	57	1,140	
7946	AMUSEMENT PARKS	18	36	
8061	HOSPITALS	349	14,650	
	TOTAL	2,278	58,102	

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TABLE 2-2. POTENTIAL INDUSTRIAL ISOPRENE EXPOSURES ESTIMATED IN THE
1981-1983 NATIONAL OCCUPATIONAL EXPOSURE SURVEY (NOES) (NIOSH, 1990)

SIC CODE	DESCRIPTION	PLANTS	TOTAL EMPLOYEES	FEMALE EMPLOYEES
2399	FABRICATED TEXTILE PRODUCTS, NEC	73	146	
2819	INDUSTRIAL INORGANIC CHEMICALS, NEC	3	119	
2822	SYNTHETIC RUBBER	15	925	108
2841	SOAP AND OTHER DETERGENTS	7	1,557	276
2911	PETROLEUM REFINING	7	105	7
7391	RESEARCH & DEVELOPMENT LABORATORIES	17	851	187
TOTAL		123	3,703	578

2.3.3 Environmental

2.3.3.1 Global Biogenic Emissions

Isoprene is emitted from plants and trees and is widely present in the environment at low concentrations (Taalman, 1996). Globally, 90% of total emissions of NMHC volatile organic compounds (VOCs) are from natural sources, primarily vegetation and forest fires. NMHC VOCs, however, constitute much smaller fractions of VOCs in industrialized areas (e.g., 16% in France), with automobiles and industrial emissions constituting the major sources of anthropogenic emissions (Dueso, 1997).

Guenther et al. (1995) estimated that the tropics emit 40 to 50% of the 503 Tg (million metric tons) [calculated as carbon] of isoprene emitted globally per year from natural sources. Other estimates have been as high as 70 to 80%. According to the authors' estimates, isoprene emissions represent 43.7% of total global natural VOC emissions. Two other recent estimates of isoprene's contribution to total global VOCs from natural sources were approximately 51%. Estimates from seven other publications from 1979 to 1992 for total global natural isoprene emissions ranged from 175 to 452 Tg C/yr (Guenther et al., 1995).

2.3.3.2 U.S. Biogenic Emissions

The chief concern in the United States with natural or biogenic emissions of NMHCs has been secondary product formation leading to the production of ozone, aerosols, and other species of concern (Altschuller, 1983). The atmospheric reactions of ambient hydrocarbons during the summer months with airborne nitrogen oxides in some areas in the United States generate enough ozone to violate the National Ambient Air Quality Standard. Even in industrialized areas such as Atlanta, GA, biogenic emissions may constitute 50% of the total atmospheric hydrocarbons in the ambient air. Considerable effort has been made to understand the contribution of isoprene since States must develop control strategies for anthropogenic emissions from stationary and mobile sources to meet the air quality standards. The Biogenic Emission Inventory Models BEIS and BEIS2 in conjunction with the Urban Airshed Model may underestimate isoprene emissions (Chang et al., 1996). An hourly biogenic emissions inventory method (PC-BEIS) is used in the U.S. EPA's regional-scale air quality models (Lamb et al., 1993).

Various techniques for predicting emissions of biogenic hydrocarbons have given substantially different estimates. The techniques must extrapolate between different locations, times, and species variations (Altschuller, 1983). Reclassifications of many landscapes from deciduous or coniferous to mixed forests, woodlands, and other categories affect the comparability with older estimates (Guenther et al., 1994). An early U.S. biogenic emissions inventory developed by Zimmerman (1979; cited by Altschuller, 1983) estimated that annual isoprene emissions in the contiguous United States totaled 15 Mg (metric tons) compared to 50 Mg of monoterpenes and 27 Mg anthropogenic emissions. Vehicular emissions represented 44% of the total anthropogenic emissions in 1977. Almost 25% of isoprene emissions and 22% of monoterpene emissions were estimated for the month of July. More recent inventories are orders of magnitude higher.

Emission rate factors for foliar emissions of isoprene, monoterpenes, and other VOCs (< 0.1 to 70, < 0.1 to 3, and < 0.5 to 5 $\mu\text{g C}$ per gram per hour, respectively) at a leaf temperature of 30 °C were recently estimated for 49 tree genera and extrapolated to the United States using land

cover composition estimates. Some species of oak (*Quercus*), *Eucalyptus*, and aspen (*Populus*) emit isoprene at even higher rates. Different woodland types have emission rate factors ranging from 0.8 to 11 mg C/m²/h. The average total biogenic emission rate factor for total VOCs in the United States is 5.1 mg C/m²/h. Isoprene concentrations in biogenic emissions range from 8% to 91% of total VOCs, with a 58% average. The average emissions of monoterpenes and other VOCs constitute 18% and 24%, respectively, of the total VOCs. Since isoprene biosynthesis is associated with photosynthesis, isoprene emissions are negligible at night (Guenther et al., 1994).

Mixed forests containing oaks (west coast, southeast, Appalachian mountains) and aspen (Rocky Mountains) have high isoprene emission factors. Spruce forests in the north central and northeast United States have moderate isoprene and high monoterpene emission factors. Pine-dominated forests in the west have low isoprene and high monoterpene emissions. For example, isoprene biogenic emissions from high isoprene emission sources such as the forests of the Appalachian and Ozark mountains are 5.5 to 8 mg/m²/h (Guenther et al., 1994).

The southern areas of the United States (EPA regions 4 [the southeastern states] and 6 [AK, LA, OK, and TX]) have the highest biogenic emissions. Of EPA regions 3-10, Regions 4 and 6 contributed 18% and 23%, respectively, of the total biogenic emissions. Summertime isoprene emissions are highest in each region and account for more than 50% of annual biogenic emissions. Highest emission rates of 2439 µg/m²/h occur in Region 6 in July and August. Land area contributors to the total annual U.S. biogenic emissions of 5.9 Tg isoprene are oak forests (2.31 Tg), other deciduous forests (1.01 Tg), coniferous forests (0.61 Tg), scrub lands (1.17 Tg) grass lands (0.49 Tg), crop lands (0.2 Tg), inland waters (0.02 Tg), and urban areas (0.08 Tg). In this inventory based on a simple forest canopy model, isoprene emissions represented 20% of total biogenic hydrocarbon emissions. The authors noted that the uncertainty in these estimates is relatively large (Lamb et al., 1993).

2.3.3.3 U.S. Anthropogenic Emissions

Sources of anthropogenic releases of isoprene to the atmosphere include ethylene production by cracking naphtha, wood pulping, oil fires, wood-burning stoves and fireplaces, other biomass combustion, tobacco smoking (3,100 µg/cigarette), gasoline, and exhaust of turbines and automobiles (HSDB, 1997).

2.3.3.4 Ambient Air

Analysis of ambient air samples from Raleigh, NC (in EPA Region 4), during August 1993, revealed that the isoprene contribution accounted for 4% of total NMHC emissions from all anthropogenic and biogenic sources. This fraction was similar to that reported in the emission inventory for North Carolina (6%) (Lawrimore and Aneja, 1997).

Atmospheric isoprene concentrations determined at sites in the Appalachian Mountains of northwestern North Carolina were wide-ranging (Seila et al., 1984), but consistent with the urban estimates. Ambient air samples taken over 13 months (September 1981 to October 1982) showed that isoprene comprised 0.16-9.8% of the total NMHCs. Essentially no isoprene was identified in meteorological and gas samples collected during the winter at four rural southeastern U.S. sites, but concentrations averaged from 9.8 to 21.15 ppb C (about 0.03 to 0.06 mg/m³) during the summer (Hagerman et al., 1997). These measurements contrasted with high

levels of several other low-molecular-weight NMHCs, which are at a maximum in the winter and decline in the summer.

Arnts and Meeks (1980) estimated that less than 10% of NMHCs in rural and remote areas is biogenic. Sites of measurements were Tulsa, OK, the Great Smoky Mountains in Tennessee, and Rio Blanco County, CO. Several other studies reviewed by Altschuller (1983) reported that the ambient air concentrations of isoprene plus monoterpenes (1 to 7 ppb C; about 0.003 to 0.02 mg/m³) accounted for only 1% to 9% of total NMHCs. During stagnation conditions, biogenic hydrocarbons may contribute more to total atmospheric hydrocarbons.

Isoprene plus monoterpene concentrations within forest canopies range from 10 to 100 ppb C, but are usually less than 10 ppb C (< 0.03 mg/m³ calculated as isoprene). Occasionally, concentrations > 100 ppb C have been reported near isoprene sources on a hot summer day and during periods of leaf drop. The much lower fraction of isoprene and monoterpenes in ambient air concentrations compared to biogenic emission inventories is apparently not due to the short lives of these reactive species or losses during sampling, storage, and analysis. Possibly, the biogenic emission inventories overestimate biogenic emissions and non-urban anthropogenic emissions inventories are underestimates (Altschuller, 1983).

2.3.4 Food and Tobacco

Foods of plant origin would be expected to be a source of daily exposure to isoprene since it is emitted by agricultural crops and is the basic structural unit in countless natural products found in foods such as terpenes and vitamins A and K (IARC, 1994). Its occurrence has been reported in the essential oil of oranges, in the fruit of hops, and in the root of carrots (Duke, 1992).

Isoprene was found to be the major component of hydrocarbons in indoor air polluted by tobacco smoke (16.7%) and in sidestream smoke (29.2%) (Barrefors and Petersson, 1993). Although baseline isoprene concentrations were the same in smokers and nonsmokers, 5 min after smoking, the concentration of isoprene in smokers' breath increased by 86% ± 26% (P < 0.001)—from 6.5 ± 0.84 (0.440 mg/m³) to 10.3 ± 1.1 nmol/L (0.701 mg/m³). The isoprene concentration declined to baseline within 15 min. The mainstream smoke concentration was 34,086 ± 3,238 nmol/L (2320 mg/m³) (Euler et al., 1996).

2.4 Regulations

EPA regulates isoprene under the Clean Air Act (CAA) and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Under the CAA, it is listed as a regulated flammable substance with a threshold quantity for accidental release prevention of 10,000 lb. Under the latter act, a final reportable quantity (RQ) of 100 lb (45.4 kg) for the compound has been established. FDA regulates isoprene as an indirect food additive in paper and paperboard components, in polymers, and in adjuvants, production aids, and sanitizers. OSHA regulates isoprene under the Hazard Communication Standard and as a hazardous chemical in laboratories.

REGULATIONS^a

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 60—PART 60—STANDARDS OF PERFORMANCE FOR NEW STATIONARY SOURCES. Promulgated: 36 FR 24877, 12/23/71. U.S. Code: 42 U.S.C. 7401, 7411, 7413, 7414, 7416, 7601 and 7602.	
	40 CFR 60—Subpart VV—Standards of Performance for Equipment Leaks of VOC in the Synthetic Organic Chemicals Manufacturing Industry. Promulgated: 48 FR 48335, 10/18/83.	This subpart applies to affected facilities in the synthetic organic chemicals manufacturing industry.
	40 CFR 60.489—Sec. 60.489 List of chemicals produced by affected facilities.	Isoprene, produced as an intermediate or final product by process units covered under this subpart, is included in this list.
	40 CFR 60—Subpart NNN—Standards of Performance for Volatile Organic Compound (VOC) Emissions From Synthetic Organic Chemical Manufacturing Industry (SOCMI) Distillation Operations. Promulgated: 55 FR 26942, 06/29/90.	This subpart applies to each affected facility designated in paragraph (b) of this section that is part of a process unit that produces any of the chemicals listed in section 60.667 as a product, co-product, by-product, or intermediate.
	40 CFR 60.667—Sec. 60.667 Chemicals affected by subpart NNN. Promulgated: 55 FR 26942, 06/29/90, as amended at 60 FR 58237 and 58238, 11/27/95.	Isoprene is included in this section.
	40 CFR 60—Subpart RRR—Standards of Performance for Volatile Organic Compound Emissions From Synthetic Organic Chemical Manufacturing Industry (SOCMI) Reactor Processes. Promulgated: 58 FR 45962, 08/31/93.	This subpart applies to each affected facility designated in paragraph (b) of this section that is part of a process unit that produces any of the chemicals listed in section 60.707 as a product, co-product, by-product, or intermediate.
	40 CFR 60.707—Sec. 60.707 Chemicals affected by subpart RRR. Promulgated: 58 FR 45962, 08/31/93, as amended at 60 FR 58238, 11/27/95.	Isoprene is included in this section.

REGULATIONS^a

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 68—PART 68—CHEMICAL ACCIDENT PREVENTION PROVISION. Promulgated: 59 FR 4493, 01/31/94. U.S. Code: 42 U.S.C. 7412(r), 7601(a)(1), 7661-7661f.	This part sets forth the list of regulated substances and thresholds, the petition process for adding or deleting substances to the list of regulated substances, the requirements for owners or operators of stationary sources concerning the prevention of accidental releases, and the State accidental release prevention programs approved under section 112(r) of the CAA.
	40 CFR 68—Subpart F—Regulated Substances for Accidental Release Prevention.	This subpart designates substances to be listed under section 112(r)(3), (4), and (5) of the CAA, as amended, identifies their threshold quantities, and establishes the requirements for petitioning to add or delete substances from the list.
	40 CFR 68.130—Sec. 68.130 List of substances.	Isoprene is listed as a regulated flammable substance; its threshold quantity for accidental release prevention is 10,000 lb.
	40 CFR 116—PART 116—DESIGNATION OF HAZARDOUS SUBSTANCES. Promulgated: 43 FR 10474, 03/13/78. U.S. Code: 33 U.S.C. 1251 et seq.	This regulation designates hazardous substances under section 311(b)(2)(A) of the FWPCA and applies to discharges of substances designated in Table 116.4.
	40 CFR 116.4—Sec. 116.4 Designation of hazardous substances. Promulgated: 43 FR 10474, 03/13/78 through 54 FR 33482, 08/14/89.	Isoprene is included in Table 116.4.
	40 CFR 117—PART 117—DETERMINATION OF REPORTABLE QUANTITIES FOR HAZARDOUS SUBSTANCES. Promulgated: 44 FR 50776, 08/29/79. U.S. Code: 33 U.S.C. 1251 et seq.	
	40 CFR 117.3—Sec. 117.3 Determination of reportable quantities. Promulgated: 50 FR 13513, 04/04/85 through 60 FR 30937, 06/12/95.	The reportable quantity established for isoprene is 100 lb (45.4 kg).

REGULATIONS^a

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 122—PART 122—EPA ADMINISTERED PERMIT PROGRAMS: THE NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM. Promulgated: 48 FR 14153, 04/01/83. U.S. Code: 33 U.S.C. 1251 et seq.	
	40 CFR 122—Subpart D—Transfer, Modification, Revocation and Reissuance, and Termination of Permits.	Isoprene is a hazardous substance required to be identified by existing dischargers if expected to be present (Appendix D).
	40 CFR 302—PART 302—DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Code: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	This part designates under section 102(a) of the CERCLA of 1980 those substances in the statutes referred to in section 101(14), identifies reportable quantities for these substances, and sets forth the notification requirements for releases of these substances. This regulation also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the CWA.
	40 CFR 302.4—Sec. 302.4 Designation of hazardous substances. Promulgated: 54 FR 33449, 08/14/89.	The statutory reportable quantity (RQ) is 1000 lb. The final RQ is 100 lb (45.4 kg).
	40 CFR 414—PART 414—ORGANIC CHEMICALS, PLASTICS, AND SYNTHETIC FIBERS. Promulgated: 52 FR 42568, 11/05/87. U.S. Codes: 33 U.S.C. 1311, 1314, 1316, 1317, and 1361.	This part applies to process wastewater discharges from all establishments or portions of establishments that manufacture the organic chemicals, plastics, and synthetic fibers (OCPSF) products or product groups covered by subparts B through H of this part.
	40 CFR 414—Subpart G—Bulk Organic Chemicals.	
	40 CFR 414.70—Sec. 414.70 Applicability; description of the bulk organic chemicals subcategory. Promulgated: 52 FR 42568, 11/05/87, as amended at 57 FR 41844, 09/11/92.	The provisions of this subpart are applicable to the process wastewater discharges resulting from the manufacture of isoprene.

REGULATIONS^a

	Regulatory Action	Effect of Regulation/Other Comments
F D A	<p>21 CFR 176—PART 176—INDIRECT FOOD ADDITIVES: PAPER AND PAPERBOARD COMPONENTS. Promulgated: 42 FR 14554, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 346, 348, 379e.</p> <p>21 CFR 176—Subpart B—Substances for Use Only as Components of Paper and Paperboard.</p> <p>21 CFR 176.180—Sec. 176.180 Components of paper and paperboard in contact with dry food. Promulgated: 42 FR 14554, 03/15/77.</p> <p>21 CFR 177—PART 177—INDIRECT FOOD ADDITIVES: POLYMERS. Promulgated: 42 FR 14572, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, and 379e.</p> <p>21 CFR 177—Subpart B—Substances for Use as Basic Components of Single and Repeated Use Food Contact Surfaces.</p> <p>21 CFR 177.1420—Sec. 177.1420 Isobutylene polymers.</p>	<p>Isoprene may be safely used as a component of the uncoated or coated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding dry food. The substance is to be used in amounts not to exceed that required to accomplish its intended physical or technical effect, and so used as to accomplish no effect in food other than that ordinarily accomplished by packaging.</p> <p>Isobutylene polymers may be safely used as components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, provided that isobutylene-isoprene copolymers produced by the copolymerization of isobutylene contain not more than 3 molar percent of isoprene.</p>

REGULATIONS^a

	Regulatory Action	Effect of Regulation/Other Comments
F D A	<p>21 CFR 178—PART 178—INDIRECT FOOD ADDITIVES: ADJUVANTS, PRODUCTION AIDS, AND SANITIZERS. Promulgated: 42 FR 14609, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, and 379e.</p> <p>21 CFR 178—Subpart D—Certain Adjuvants and Production Aids.</p> <p>21 CFR 178.3850—Sec. 178.3850 Reinforced wax. Promulgated: 42 FR 14609, 03/15/77, as amended at 47 FR 1288, 01/12/82.</p>	<p>The substances and optional adjuvant substances employed in the production of or added to reinforced wax include the copolymer of isobutylene modified with isoprene.</p>
O S H A	<p>29 CFR 1910—PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS. Promulgated: 39 FR 23502, 06/27/74.</p> <p>29 CFR 1910.1200—Sec. 1910.1200 Hazard Communication. Promulgated: 61 FR 9245, 03/07/96. U.S. Code: 29 U.S.C. 653, 655, and 657; 5 U.S.C. 553.</p> <p>20 CFR 1910.1450—Sec. 1910.1450 Occupational exposure to hazardous chemicals in laboratories. Promulgated: 61 FR 5508, 02/13/96. OSH Act. Final Rule.</p>	<p>This section requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication Program to include labels, materials safety data sheets, and worker training.</p> <p>As a select carcinogen (IARC Group 2B, possibly carcinogenic to humans, and now NTP-listed as reasonably anticipated to be a human carcinogen), isoprene is included as a chemical hazard in laboratories. Employers are required to provide employee information and training and a Chemical Hygiene Plan.</p>

^aThe regulations in this table have been updated through the 1998 issues of Federal Code of Regulations titles 21, 29, and 40.

3.0 HUMAN STUDIES

Studies Evaluated by IARC (1994) and after IARC (1994)

A March 1998 online literature search of six biomedical databases and current awareness searches through August 1998 did not identify any studies of the effects of exposure to isoprene on humans. None were described by IARC (1994).

4.0 EXPERIMENTAL CARCINOGENICITY

4.1 Animal Studies Reviewed by IARC (1994)

IARC (1994) evaluated a draft of an NTP study (Melnick et al., 1994) with male rats and male mice designed to determine if isoprene produces a carcinogenic response after intermediate exposure durations. In this study, groups of 40 male rats (F344/N) and 40 male mice (B6C3F₁) were exposed to isoprene by inhalation of 0 (chamber control), 70, 220, 700, 2200, or 7000 ppm for 6 h/day on 5 days/wk for 6 mo followed by a 6-mo recovery period without exposure to isoprene (stop-exposure protocol). Ten animals per group were killed at the end of the exposure period and examined for exposure-related effects.

Rats had an increased incidence and severity of interstitial cell hyperplasia of the testis after 6 mo of exposure to 7000 ppm isoprene. Following the 6-mo recovery period, slightly greater incidences of interstitial cell adenoma of the testes were observed in rats that had been exposed to 700, 2200, or 7000 ppm (3/30 [control], 7/30, 8/29, 9/30, respectively; trend test: $p = 0.02$). Survival was unaffected by isoprene exposure.

The IARC Working Group considered the rat study to be inadequate for assessment of carcinogenicity because of the short duration of the study and the high spontaneous incidence of interstitial cell tumors in this strain of rat at two years.

Mice, after exposure to 700 ppm or higher concentrations of isoprene for 6 mo, had increased incidences of neoplasms at four organ sites when examined after the 6-mo recovery. Compared to the control group, neoplasms in groups exposed to 700, 2200, or 7000 ppm occurred at a significantly greater incidence in the liver (7/30 [control], 15/30, 18/30, 17/28, respectively), lungs (2/30 [control], 5/30, 10/30, 9/28, respectively), forestomach (0/30 [control], 1/30, 4/30, 6/30, respectively), and Harderian gland (2/30 [control], 14/30, 13/30, 12/30, respectively). Incidences of multiple neoplasms and/or malignant neoplasms were also increased in exposed groups compared to controls. Survival was decreased in the highest (7000 ppm) exposure group.

The IARC Working Group concluded that the mouse study provided sufficient evidence in experimental animals for the carcinogenicity of isoprene.

4.2 Studies Post-IARC (1994)

Experimental animals, methods, and cancer incidences are presented in Table 4.1. A chronic inhalation study with mice evaluated three independent variables: concentration, length of daily exposure, and number of weeks of exposure (Placke et al., 1996). Male B6C3F₁ mice were exposed to isoprene for 5 days/wk, 8 h/day or 4 h/day, for 20, 40, or 80 wk, so that the concentration x time (duration of exposure) values provided a series of theoretically equivalent exposure hazards. Exposure to isoprene produced increased incidences of neoplasms at multiple organ sites. However, the duration of exposure did not appear to predict tumor risk at any site.

For groups of 50 males, the 8 h/day exposure levels, expressed as ppm-wk, were 0-80, 10-80, 70-40, 70-80, 140-40, 280-20, 280-80, 700-80, 2200-40, and 2200-80. The 4 h/day ppm-wk exposures for two additional groups were 2200-20 and 2200-80. Groups were held for 96 or 105 wk. Significant ($p < 0.05$) increases were observed in the incidence of liver adenoma, liver carcinoma, primary alveolar and bronchiolar adenomas, lung carcinoma, Harderian gland adenomas, and histiocytic sarcoma.

In one part of this study, groups of female mice were exposed to 0, 10, or 70 ppm isoprene 8 h/day for 80 wk only. The 70-ppm group had a significant increase ($p < 0.05$) in the incidence of Harderian gland adenoma (2/49 [control], 8/49) and pituitary adenoma (1/49 [control], 9/49). Based on comparison to historical controls, this increase may have been related to isoprene exposure.

In a rat study, isoprene was found to be carcinogenic to the kidney and testes of males and to the mammary gland of males and females. Groups of 50 male and 50 female F344/N rats were exposed to 0, 220, 700, or 7000 ppm isoprene by inhalation, 6 h/day, 5 days/w for two years (NTP TR 486, 1997 draft). Survival and mean body weights of exposed males and females were similar to those of chamber controls.

In groups of males exposed to 7000 ppm isoprene, there was a significant increase in the incidence of fibroadenoma versus the chamber control groups (2/50 [control], 21/50). The incidence of multiple fibroadenoma was also increased in this exposure group (1/50 [control], 7/50; $p \leq 0.05$). Although the incidences of fibroadenoma in the male rats exposed to 220 and 700 ppm isoprene were not significantly greater than the chamber control group (2/50 [control], 4/50, 6/50, respectively), they exceeded the historical control ranges for this neoplasm (0-6%). In addition, the finding of mammary gland carcinoma in exposed male rats (0/50 [control], 1/50 [220 ppm], 1/50 [700 ppm], 2/50 [7000 ppm], respectively) is noteworthy because these neoplasms rarely occur in control male rats (1/950). In females exposed to 220, 700, or 7000 ppm isoprene, the incidence of mammary gland fibroadenoma (19/50 [control], 35/50, 32/50, 32/50, respectively) and multiple fibroadenoma (7/50 [control], 12/50, 19/50, 17/50, respectively) was significantly increased.

A significant increase in the incidence of renal tubule adenoma or carcinoma occurred in groups of male rats exposed to 700 or 7000 ppm isoprene (2/50 [control], 6/50, 8/50, respectively). The incidence of renal tubule hyperplasia, thought to represent an early stage in the development of renal carcinoma and adenoma, was also significantly increased in these groups.

Exposure-related increases in the incidence of testicular interstitial cell adenoma in the 700- and 7000-ppm groups (33/50 [control], 44/50, 48/50, respectively) significantly exceeded the high spontaneous incidence of this adenoma in F344/N rats. The significantly increased incidence of bilateral testicular interstitial cell adenoma observed in the 700- and 7000-ppm groups (20/50 [control], 37/50, 48/50, respectively) support the contention that exposure to isoprene increased the incidence of these neoplasms.

A low incidence of rare brain neoplasms including benign astrocytoma, malignant glioma, malignant medulloblastoma, benign granular cell tumor, and meningeal sarcoma, was seen in exposed females and may have been due to isoprene exposure.

Table 4-1. Experimental Carcinogenicity Studies with Isoprene (Post-IARC, 1994)

Age, Strain, Species	No. and Sex Exposed	No. and Sex Controls	Chemical Form and Purity	Dose and Route	Duration of Exposure	Results/Comments	Reference
<i>Mice</i>							
10 wk B6C3F ₁ mice	eleven groups of 50 males	one group of 50 males	gaseous isoprene ≥ 99% purity	males exposed by inhalation in test chamber to 0, 10, 70, 140, 280, 700, or 2200 ppm	males exposed 4 or 8 h/day, 5 days/wk for 20, 40, or 80 wk The 8 h/day exposure levels, expressed as ppm-wk, were 0-80, 10-80, 70-40, 70-80, 140-40, 280-20, 280-80, 700-80, 2200-40, and 2200-80. The 4 h/day ppm-wk exposures for two additional groups were 2200-20 and 2200-80. Groups were held for 96 or 105 wk.	NEOPLASM INCIDENCE IN MALES (p < 0.05) <u>Liver adenoma</u> 11/50 [control], 22/50, 18/49, 24/50, 27/48, 28/47, 30/50 at exposures* of 0-80, 140-40, 280-20, 280-80, 700-80, 2200-40, 2200-80 ppm-wk for 8 h/day. 11/50 [control], 22/50, 21/50 at exposures of 0-80, 2200-20 and 2200-80 ppm-wk for 4 h/day <u>Liver carcinoma</u> 9/50 [control], 10/50, 12/49, 16/50, 17/48, 18/47, 16/50 at exposures of 0-80, 140-40, 280-20, 280-80, 700-80, 2200-40, and 2200-80 ppm-wk for 8 h/day. 9/50 [control], 12/50, 15/50 at exposures of 0-80, 2200-20 and 2200-80 for 4 h/day <u>Lung primary alveolar & bronchiolar adenoma</u> 11/50 [control], 23/50, 29/49, 30/50 at exposures of 0-80, 700-80, 2200-40, and 2200-80 for 8 h/day <u>Lung carcinoma</u> 0/50 [control], 7/50, 3/49, 7/50 at exposures of 0-80, 700-80, 2200-40, and 2200-80 for 8 h/day <u>Harderian gland adenoma</u> 4/47 [control], 12/50, 16/49, 17/50, 26/49, 31/49, 35/50 at exposures of 0-80, 140-40, 280-20, 280-80, 700-80, 2200-40, 2200-80 ppm-wk for 8 h/day 4/47 [control], 19/49 and 28/50 at exposures of 0-80, 2200-20 and 2200-80 ppm-wk for 4 h/day	Placke et al. (1996)

Table 4-1. Experimental Carcinogenicity Studies with Isoprene (Post-IARC, 1994) (Continued)

Age, Strain, Species	No. and Sex Exposed	No. and Sex Controls	Chemical Form and Purity	Dose and Route	Duration of Exposure	Results/Comments	Reference
10 wk B6C3F1 mice	two groups of 50 females	one group of 50 females	gaseous isoprene $\geq 99\%$ purity	females exposed by inhalation in test chamber to 0, 10, or 70 ppm	females exposed 8 h/day, 5 days/wk for 80 wk	<p>NEOPLASM INCIDENCE IN MALES ($p < 0.05$) (cont'd.) Histiocytic sarcoma 0/50 [control], 8/50 at exposures of 0-80, 280-20 ppm-wk for 8 h/day 0/50 [control], 7/50 at exposures of 0-80, 2200-80 ppm-wk for 4 h/day</p> <p>NEOPLASM INCIDENCE IN FEMALES ($p < 0.05$) Harderian gland adenoma 2/49 [control], 8/49 at exposures of 0-80, 70-80 ppm-wk for 8 h/day Pituitary adenoma 1/49 [control], 9/49 in group at exposures of 0-80, 70-80 ppm</p> <p>Concentration-related reduction in survival - groups exposed to 280-2200 ppm at wk 80 had $< 50\%$ survival compared to controls or lower concentration groups, animals necropsied at wk 96</p>	Placke et al. (1996)

Table 4-1. Experimental Carcinogenicity Studies with Isoprene (Post-IARC, 1994) (Continued)

Age, Strain, Species	No. and Sex Exposed	No. and Sex Controls	Chemical Form and Purity	Dose and Route	Duration of Exposure	Results/Comments	Reference
<i>Rats</i>							
6 wk F344/N rats	groups of 50 M and 50 F	50 M; 50 F	isoprene vapor; purity > 99%	220, 700, or 7000 ppm by inhalation	6 h/day, 5 days/wk for 104 wk	<p>NEOPLASM INCIDENCE IN MALES (p < 0.05)</p> <p><u>Mammary gland fibroadenoma</u> 2/50 [control], 4/50, 6/50, 21/50 at exposures of 220, 700, or 7000 ppm, respectively</p> <p><u>Mammary gland fibroadenoma or carcinoma (combined)</u> 2/50 [control], 5/50, 7/50, 21/50 at exposures 220, 700, or 7000 ppm, respectively</p> <p><u>Mammary gland multiple fibroadenoma</u> 1/50 [control], 7/50 at exposures of 7000 ppm</p> <p><u>Renal tubule adenoma or carcinoma</u> 2/50 [control], 8/50, 15/50 at exposures of 700 or 7000 ppm, respectively</p> <p><u>Testicular interstitial cell adenoma</u> 33/50 [control], 37/50, 44/50, 48/50 at exposures of 220, 700, or 7000 ppm, respectively</p> <p><u>Testicular bilateral interstitial cell adenoma</u> 20/50 [control], 29/50, 37/50, 48/50 at exposures of 220, 700, or 7000 ppm, respectively</p> <p>NEOPLASM INCIDENCE IN FEMALES (p < 0.05)</p> <p><u>Mammary gland fibroadenoma</u> 19/50 [control], 35/50, 32/50, 32/50 at exposures of 220, 700, or 7000 ppm, respectively</p> <p><u>Mammary gland multiple fibroadenoma</u> 7/50 [control], 12/50, 19/50, 17/50 at exposures of 220, 700, or 7000 ppm, respectively</p>	NTP TR 486 (1997 draft)

* #.# = ppm-wk; daily exposure 8 h unless otherwise noted

5.0 GENOTOXICITY

Studies on the genotoxic effects of isoprene have been reviewed by IARC (1994) and NTP (1995, 1997 draft).

5.1 Genotoxicity Studies Reviewed by IARC (1994)

In bacterial systems, isoprene did not induce reverse mutations in *Salmonella typhimurium* strains TA98, TA100, TA1530, TA1535, TA1537 and TA1538 tested in the presence and absence of metabolic activation (de Meester et al., 1981; Mortelmans et al., 1986; both cited by IARC, 1994). The monoepoxide intermediates 3,4-epoxy-2-methyl-1-butene (EPOX-I) and 3,4-epoxy-2-methyl-1-butene (EPOX-II) were negative in TA98 and TA100, whereas the diepoxide intermediate was a potent mutagen in TA100. It should be noted that the lack of mutagenicity of isoprene may be an artifact of the preincubation protocol, which may have resulted in evaporation and/or incomplete biotransformation, and the monoepoxide EPOX-I may have been rapidly hydrolyzed, thus precluding detection of EPOX-I genetic toxicity in *S. typhimurium* (NTP, 1997 draft).

In vivo, isoprene induced a significant increase in sister chromatid exchange (SCE) in bone marrow and micronuclei in erythrocytes of male B6C3F₁ mice exposed to isoprene at concentrations of 438, 1750, or 7000 ppm via inhalation for 12 days (6 h/day), but an increase in chromosome aberrations was not observed in the bone marrow of the same mice (Tice et al., 1988).

5.2 Additional Genotoxicity Studies Summarized in NTP (1995, 1997 draft)

In vitro assays of isoprene with cultured Chinese hamster ovary (CHO) cells showed no increase in SCE or chromosomal aberrations, in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix (Galloway et al., 1987; cited by NTP, 1995; NTP, 1997 draft). Isoprene concentrations in these tests may have been reduced by evaporation (NTP, 1997 draft).

In vivo genetic toxicity assays with isoprene gave positive results. Male and female mice exposed to isoprene for 13 weeks had significantly increased frequencies of micronuclei in polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) (Jauhar et al., 1988; cited by NTP, 1997 draft). Male and female rats exposed to isoprene by inhalation for 4 weeks showed no significant increase in the frequency of micronucleated lung fibroblasts (NTP, 1997 draft).

5.3 Genetic Mutations in Isoprene-Induced Tumors

An analysis of *ras* protooncogenes in tumors from mice chronically exposed to isoprene indicated that specific mutations contribute to tumorigenesis (Hong et al., 1997). The tumors were Harderian gland tumors from male B6C3F₁ mice given two doses of isoprene (2200 or 7000 ppm) by inhalation (6 h/day; 5 days/wk) for 26 weeks, followed by a 26-week recovery. Mutations in these tumors were compared to mutations in Harderian gland tumors from mice unexposed to isoprene (negative control) and to mutations in 1,3-butadiene-induced Harderian gland tumors (positive control). A higher frequency of *ras* mutations occurred in isoprene-induced tumors than in tumors from the positive or negative controls. Most of the mutations were A→T transversions at K-*ras* codon 61 and C→A transversions at H-*ras* codon 61.

6.0 OTHER RELEVANT DATA

6.1 Absorption, Distribution, Metabolism, and Excretion in Humans

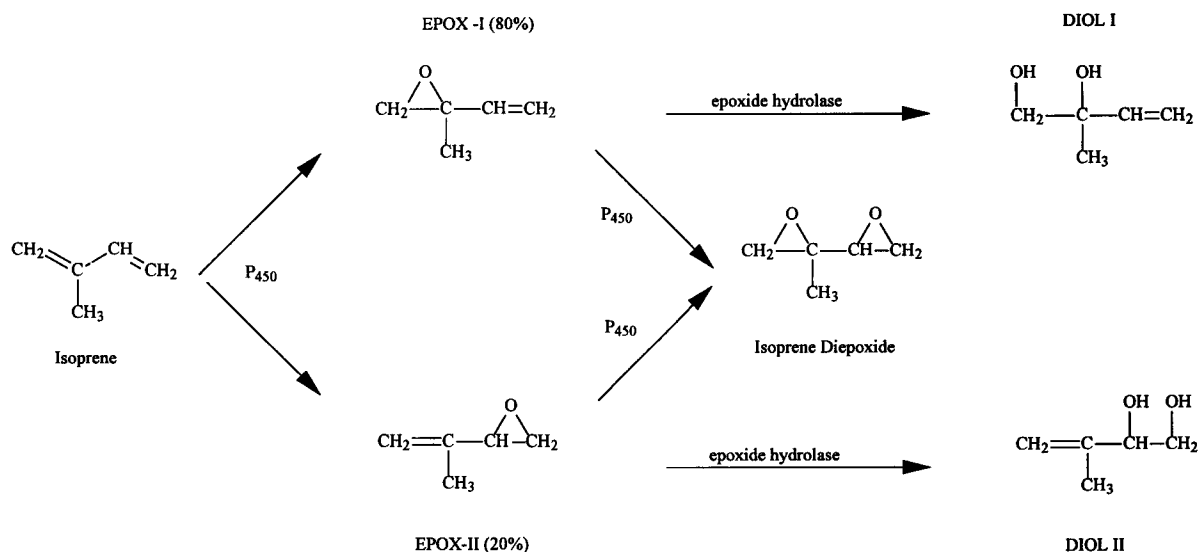
Isoprene is probably an endogenous precursor of cholesterol (Deneris et al., 1984; cited by IARC, 1994). Concentrations were reported to range from 15 to 70 nmol/L in human blood (Cailleux et al., 1992; cited by IARC, 1994) and from 10 to 30 nmol/L (0.68 to 2.0 mg/m³) in human breath (Cailleux and Allain, 1989; cited by IARC, 1994).

The metabolism of isoprene and two isoprene monoepoxides (Fig. 1) was explored with microsomes derived from cell lines expressing eight human cytochrome P450 enzymes and with liver microsomes from humans, rats, and mice (Bogaards et al., 1996). The single human enzymes were CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4. The enzyme CYP2E1 was associated with the highest formation rate of the two isoprene monoepoxides and with the formation of the diepoxide from each of the monoepoxide intermediates (Fig. 1). Results from incubation of human liver microsomal preparations were consistent with those obtained from tests with single human enzymes. In the presence of inhibitor, rates of monoepoxide formation were similar in human, mouse, and rat liver microsomes; however, without inhibitor, the total amount of monoepoxides present at the end of the incubation period was highest for mouse liver microsomes.

6.2 Absorption, Distribution, Metabolism, and Excretion in Experimental Systems

The rate of endogenous isoprene production was 1.9 µmol/kg/h in rats and 0.4 µmol/kg/h in mice (Peter et al., 1987; cited by NTP, 1997 draft). Rats exposed 6 hours to radiolabeled isoprene by nose-only inhalation at concentrations of 8, 260, 1480, or 8200 ppm (23, 738, 4200, 23,268 mg/m³) retained 19, 9, 6, or 5%, respectively (Dahl et al., 1987; cited by IARC, 1994). Mice showed steady-state levels of 25 to 6800 ng/mL (0.0004 to 0.1 µmol/mL) in blood after inhalation exposure to 20 to 2000 ppm isoprene (Bond et al., 1991; cited by IARC, 1994). In rats, most of the inhaled isoprene and metabolites was distributed to liver, blood, and fat (Dahl et al., 1987; cited by IARC, 1994).

Isoprene was metabolized to two monoepoxides (Fig. 6-1) by liver microsomes from mice, rats, rabbits, hamsters, and humans; these metabolites may be hydrolyzed, conjugated with glutathione, or further oxidized to isoprene diepoxide (Del Monte et al., 1985; Gervasi and Longo, 1990; Wistuba et al., 1994; all cited by NTP, 1997 draft; Bogaards et al., 1996). In rats exposed to isoprene via inhalation, volatile metabolites in respiratory tract tissue suggested significant metabolism of isoprene in the respiratory tract (Dahl et al., 1987; cited by IARC, 1994). The maximal metabolic velocity (V_{max}) for diepoxide formation was 6-fold greater in microsomes from mice and Syrian hamsters than the V_{max} in microsomes from rats and rabbits (Longo et al., 1985; cited by IARC, 1994), indicative of significant species differences in metabolism.

Figure 6-1. Metabolism of Isoprene in the Liver of Rabbits, Hamsters, Rats, and Mice.

[Excerpt from NTP, 1997 draft; Original Source: Gervasi and Longo, 1990]

EPOX I = 3,4-epoxy-3-methyl-1-butene; EPOX II = 3,4-epoxy-2-methyl-1-butene

Comparative studies with mammalian *in vitro* systems indicate that there are significant stereochemical and mechanistic differences among species (Small et al., 1997). Enantiomers of the monoepoxide 2-(1-methylethenyl)oxirane were identified in liver microsome preparations from rats, mice, rabbits, dogs, monkeys, and humans. Rats preferentially formed (*S*)-2-(1-methylethenyl)oxirane compared with the (*R*)-enantiomer, whereas microsomes from dog, monkey, or male human preferentially formed (*R*)-2-(1-methylethenyl)oxirane. Metabolites from isoprene incubated with human female microsomes did not show enantioselectivity.

Studies of the metabolism of isoprene indicate formation of a diepoxide from both monoepoxides (Fig. 1) in all mammalian systems (Small et al., 1997; Wistuba et al., 1994; Bogaards et al., 1996; both cited by NTP, 1997 draft). It should be noted that data were obtained mostly from *in vitro* studies.

About 75% of the retained isoprene radioactivity, delivered to rats as described in the first paragraph of this subsection, was excreted in urine within 66 hours (Dahl et al, 1987; cited by IARC, 1994). The maximal metabolic elimination rates for isoprene inhaled by rats and mice were estimated to be 130 and 400 $\mu\text{mol/kg/h}$, respectively (Peter et al., 1987; cited by IARC, 1994).

A chemical study of the isoprene monoepoxide EPOX-I reactivity with nucleophiles found a relatively high $\text{S}_{\text{N}}2$ reactivity at C-3, leading to efficient adduct formation (Bleasdale et al., 1996).

6.3 Structure-Activity Relationships (SAR)

Isoprene is the 2-methyl analogue of 1,3-butadiene, while chloroprene is the 2-chloro analogue of 1,3-butadiene. Chloroprene is also an analogue of vinyl chloride. The three structural analogues of isoprene are animal carcinogens and known (1,3-butadiene, vinyl chloride) or reasonably anticipated to be (chloroprene) human carcinogens.

6.3.1 1,3-Butadiene

Based on the human epidemiological and rodent carcinogenicity data published prior to 1992, IARC (1992) concluded that there was sufficient evidence for the carcinogenicity of 1,3-butadiene in experimental animals and limited evidence for its carcinogenicity in humans. The results of more recent mechanistic and epidemiological studies strengthened the link between occupational exposure to 1,3-butadiene and cancer, with the result that NTP has classified 1,3-butadiene as *known to be a human carcinogen* (NTP, 1999 [Report on Carcinogens, 9th ed]). The increased mortality risk in humans occupationally exposed to 1,3-butadiene is largely for leukemia, lymphosarcoma, and reticulosarcoma (IARC, 1992; West et al., 1995; Ward et al., 1996; Divine and Hartman, 1996; Macaluso et al., 1996; Delzell et al., 1996).

1,3-Butadiene is a multi-species, multi-organ carcinogen (IARC, 1992; NTP, 1993; Melnick et al., 1994). In the NTP inhalation bioassay (NTP, 1993), 1,3-butadiene induced significant increases in the incidence of hemangiosarcomas of the heart, malignant lymphomas, alveolar/bronchiolar adenomas and carcinomas, squamous cell papillomas or carcinomas of the forestomach, Harderian gland adenomas, hepatocellular adenomas or carcinomas, mammary-gland carcinomas, and granulosa-cell tumors of the ovary in mice. In a rat inhalation study, 1,3-butadiene induced an increased incidence of pancreatic exocrine neoplasms and Leydig cell tumors of the testis in males, and uterine stromal sarcomas, Zymbal's gland carcinomas, thyroid follicular-cell neoplasms, and mammary gland neoplasms in females (Owen et al., 1987).

Based on generally positive results in a variety of short-term *in vitro* and *in vivo* genotoxicity studies, 1,3-butadiene would be classified as a genotoxic carcinogen (IARC, 1992; NTP, 1993). 1,3-Butadiene was positive for the induction of gene mutations in *S. typhimurium*, but not in mouse lymphoma cells, in both the presence and absence of metabolic activation. It was negative for the induction of both wing spot and sex-linked recessive lethal mutations in *D. melanogaster*. In mammalian systems *in vitro*, 1,3-butadiene was weakly positive for the induction of SCE in Chinese hamster ovary cells with metabolic activation, while reported as negative and positive for SCE induction in human lymphocytes (conflicting studies) with and without metabolic activation. Of most relevance, 1,3-butadiene was positive *in vivo* for the induction of DNA-DNA and DNA-protein cross-links in liver and lung of mice, but not rats, and in mice for the induction of dominant lethal mutations and sperm abnormalities, SCE and chromosomal damage in bone marrow cells, and micronucleated erythrocytes measured in peripheral blood. In occupationally exposed humans, 1,3-butadiene was positive for the induction of hemoglobin adducts, negative for SCE in lymphocytes, and reported as both positive and negative (conflicting studies) for *hprt* mutations and chromosomal aberrations in lymphocytes.

6.3.2 Chloroprene

Chloroprene is *reasonably anticipated to be a human carcinogen* based on evidence of benign and malignant tumor formation at multiple tissue sites in multiple species of experimental animals (NTP, 1998). Inhalation exposure of rats to chloroprene vapors induced increased incidences of neoplasms of the oral cavity, thyroid gland, and kidney in males and females, neoplasms of the lung in males, and neoplasms of the mammary gland in females. Inhalation exposure of mice to chloroprene vapors induced increased incidences of neoplasms of the lung, circulatory system (hemangiomas and hemangiosarcomas), forestomach, and Harderian gland in males and females, kidney neoplasms in males, and neoplasms of the mammary gland, liver, Zymbal's gland, skin, and mesentery in females.

There is limited evidence for the carcinogenicity of chloroprene in humans. Data from two studies suggest that occupational exposure to chloroprene may increase cancer risk for digestive and lymphatic/hematopoietic tumors (Pell, 1978, cited by IARC 1979a) and for liver, lung, and lymphatic tumors (Li et al., 1989). One case of liver angiosarcoma was reported in a worker with no known occupational exposure to vinyl chloride who had extensive exposure to polychloroprene (which may contain 0.5% chloroprene) (Infante, 1977).

Recent studies on the genotoxicity of chloroprene have been uniformly negative; conflicting results with earlier studies have been attributed to differences in the age and purity of the test samples. Positive mutagenicity results of chloroprene in bacteria (Bartsch et al., 1975; 1979) were considered to be due to cyclic dimers that accumulate in aged samples (Westphal et al., 1994). At the exposure concentrations used in the cancer inhalation studies, chloroprene did not induce sister chromatid exchanges or chromosomal aberrations in mouse bone marrow cells nor did it increase the frequency of micronucleated erythrocytes in peripheral blood (Tice et al., 1988). Oxidation of chloroprene to epoxide intermediates has been postulated to occur based on the detection of alkylated derivatives of the trapping agent 4-(4-nitrobenzyl)pyridine in incubations of chloroprene and mouse liver microsomes (Bartsch et al., 1979). Chloroprene-induced lung and Harderian gland neoplasms in mice had a high frequency of unique *K-ras* mutations (NTP, 1998). Chloroprene induced all of the types of tumors that were induced by 1,3-butadiene in mice except for lymphomas and ovarian neoplasms.

6.3.3 Vinyl Chloride

Based on human epidemiological studies and case reports and rodent carcinogenicity data, IARC (1979b) concluded that there was sufficient evidence for the carcinogenicity of vinyl chloride to humans and experimental animals. IARC (1987) reaffirmed vinyl chloride's evaluation as a human carcinogen, citing several additional epidemiological studies and case reports. IARC (1987) and Green (1990) confirmed a causal association between occupational exposure to vinyl chloride and angiosarcoma of the liver, hepatocellular carcinoma, brain and lung tumors, and malignancy of the hematopoietic and lymphatic system. Some studies (IARC, 1987) indicated a possibility of increased risk of gastric, liver, and gastrointestinal cancer. Green (1990) noted that workers in vinyl chloride manufacturing also experienced increases in tumors of the skin and thyroid, though a causal relationship was not established. One epidemiological study indicated excessive fetal mortality among wives of workers exposed to vinyl chloride, and several others reported increased rates of birth defects in children whose parents lived in communities with vinyl chloride-poly(vinyl chloride) or other chemical processing facilities (IARC, 1979b). Two proportionate mortality studies of deceased workers who had been

involved in plastics fabrication suggested increases in cancer of the digestive system (both sexes) and increases in cancer of the urinary system and breast in women (IARC, 1979b).

Vinyl chloride has been extensively tested in rats, hamsters, and mice via inhalation exposure and oral, subcutaneous, and intraperitoneal administration. Oral administration or inhalation of vinyl chloride induced Zymbal gland tumors in rats and hamsters, nephroblastomas in rats, forestomach papillomas and melanomas in hamsters, and pulmonary and mammary gland tumors in mice (IARC, 1979b; 1987). In all three species, exposure to vinyl chloride induced hemangiosarcoma of the liver (IARC, 1979b; 1987; Green, 1990). Vinyl chloride was carcinogenic in rats exposed prenatally (IARC, 1979b).

Green (1990) observed that vinyl chloride's wide range of effects in many species was characteristic of a genotoxic carcinogen. Workers exposed to vinyl chloride vapor showed induction of chromosomal aberrations in peripheral blood lymphocytes (IARC, 1987). Two additional studies of exposed workers indicated negative results for SCE, while one study indicated a weakly positive response (IARC, 1987). In rodents exposed *in vivo*, vinyl chloride induced chromosomal aberrations, SCE, and micronuclei in bone marrow cells, and alkylated DNA in tissues of mice and rats (IARC, 1987). *In vitro*, vinyl chloride induced unscheduled DNA synthesis in rat hepatocytes, gene mutation in Chinese hamster lung cells, gene conversion in yeast, and DNA damage and mutation in bacteria. It also induced sex-linked recessive lethals in *Drosophila* and was mutagenic in plants and *Schizosaccharomyces pombe* (but not other fungi) (IARC, 1987). Green (1990) suggested that vinyl chloride's carcinogenic activity results from its metabolism by microsomal mixed-function oxidases to chloro-oxirane (chloroethylene oxide) and chloroacetaldehyde, two mutagenic metabolites, and concluded that vinyl chloride is a classical genotoxin causing cancer by somatic mutation.

7.0 MECHANISMS OF CARCINOGENESIS

Isoprene was tested for carcinogenicity because of its structural similarity to 1,3-butadiene, an animal and human carcinogen, and because of the potential for human exposure (NTP, 1997 draft). Bioassays with rats and mice investigated cancer occurrence after 26 weeks of inhalation exposure followed by 26 weeks of recovery and after 2 years of inhalation exposure (see Section 4), respectively. Results indicated that isoprene is clearly carcinogenic in both species. There are several common sites of tumor induction by 1,3-butadiene and isoprene and some sites that are specific for each chemical (Table 7-1).

TABLE 7-1. Summary of Sites of Increased Incidences of Neoplasms in 2-Year Inhalation Studies of Isoprene and 1,3-Butadiene in Male and Female Rats and Mice

	Isoprene	1,3-Butadiene
Rats	Kidney (M) Mammary Gland (M, F) Testis (M)	Brain (M) Mammary Gland (F) Pancreas (M) Testis (M) Thyroid Gland (F) Uterus (F) Zymbal's Gland (F)
Mice	Circulatory System (Heart and Spleen Hemangiosarcoma) (M, F) Forestomach (M) Harderian Gland (M, F) Hematopoietic System (M) Liver (M) Lung (M) Pituitary Gland (F)	Circulatory System (Heart and Spleen Hemangiosarcoma) (M,F) Forestomach (M) Harderian Gland (M, F) Hematopoietic System (M, F) Kidney (M) Liver (M) Lung (M, F) Mammary Gland (F) Ovary (F) Preputial Gland (M)

Adapted from NTP (1997 draft)

As with 1,3-butadiene, reactive alkylating metabolites of isoprene may play a role in carcinogenesis, although other mechanisms must also be considered. Isoprene and 1,3-butadiene are metabolized to mono- and diepoxide intermediates by microsomal cytochrome P450-dependent monooxygenases (CYP2E1) (Del Monte et al., 1985; Longo et al., 1985; Malvoisin et al., 1979; Malvoisin and Roberfroid, 1982; all cited by NTP, 1997 draft; Bogaards et al., 1996). Detoxification of these epoxide intermediates may occur by hydrolysis catalyzed by epoxide hydrolase or conjugation with glutathione catalyzed by glutathione-S-transferase. The diepoxide intermediates of both compounds were mutagenic in *S. typhimurium*; however, unlike the monoepoxide intermediates of isoprene, the monoepoxide intermediate of 1,3-butadiene biotransformation was also mutagenic in the *Salmonella* assay (Gervasi et al., 1985; cited by NTP, 1997 draft). Bleasdale et al. (1996) recently reported that EPOX I is highly reactive with amino and thiolate nucleophiles via an S_N2 mechanism. Therefore, the lack of observed mutagenicity of this epoxide in *S. typhimurium* may be an artifact of the incubation protocol and the high spontaneous hydrolysis rate of this reactive compound (NTP, 1997 draft).

In mice, isoprene and 1,3-butadiene induced SCE in bone marrow cells and increased the frequency of micronucleated erythrocytes in peripheral blood (Tice et al., 1987; cited by NTP, 1997 draft; Tice et al., 1988). Unlike isoprene, 1,3-butadiene also induced chromosomal aberrations in bone marrow cells of mice. Neither compound has been shown to be genotoxic in rats. The above findings, as well as the identification of unique oncogene mutations in

neoplasms of mice exposed to isoprene, are consistent with a genotoxic mechanism of tumor induction.

Activation of *K-ras*, predominantly by an A→T transversion at codon 61, appears to be involved in the induction of Harderian gland neoplasms (Hong et al., 1997).

Evaluations of dose-response relationships for isoprene-induced neoplasms in rats indicated that the carcinogenic effects in the kidney are probably mediated by the epoxide intermediates (NTP, 1997 draft). Carcinogenic effects in the mammary gland of rats may involve a combination of effects from isoprene epoxides and the parent compound with possible hormonal influences.

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APPENDIX A

**Excerpt from IARC Monograph (1994)
Volume 60; pp. 215-232**

ISOPRENE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 78-79-5

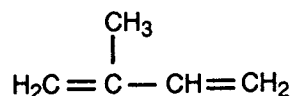
Deleted CAS Reg. No.: 78006-92-5

Chem. Abstr. Name: 2-Methyl-1,3-butadiene

IUPAC Systematic Name: 2-Methyl-1,3-butadiene

Synonyms: Isopentadiene; β -methylbivinyll; 2-methylbutadiene; 3-methyl-1,3-butadiene

1.1.2 Structural and molecular formulae and relative molecular mass



C_5H_8

Relative molecular mass: 68.12

1.1.3 Chemical and physical properties of the pure substance

- (a) *Description*: Colourless liquid or gas with faint aromatic odour (Exxon Chemical Co., 1989; Weitz & Loser, 1989)
- (b) *Boiling-point*: 34 °C (Lide, 1991)
- (c) *Melting-point*: -146 °C (Lide, 1991)
- (d) *Density (liquid)*: 0.6810 at 20 °C/4 °C (Lide, 1991)
- (e) *Spectroscopy data*: Infrared [prism (688); grating (154)], ultraviolet and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991; US National Library of Medicine, 1993a).
- (f) *Solubility*: Insoluble in water (0.029 mol%); soluble in acetone, benzene, diethyl ether and ethanol (Lide, 1991)
- (g) *Volatility*: Vapour pressure, 60.7 kPa at 20 °C (Weitz & Loser, 1989); relative vapour density (air = 1), 2.35 (Exxon Chemical Co., 1989)
- (h) *Stability*: Lower explosive limit, 1-1.5% (28-40 g/m³) (Weitz & Loser, 1989); will ignite readily at ambient temperatures; will polymerize vigorously or decompose with shocks of pressure or temperature (Exxon Chemical Co., 1989)

- (i) *Octanol-water partition coefficient (P)*: log P, 2.30 (US National Library of Medicine, 1993a)
- (j) *Conversion factor*: $\text{mg/m}^3 = 2.79 \times \text{ppm}^a$

1.1.4 Technical products and impurities

Isoprene is available commercially at a minimal purity of 99.0%; minor contaminants include acetylenes, cyclopentadiene, peroxides, piperylenes, pentenes and sulfur. It is typically inhibited with *para-tert-butyl* catechol (Exxon Chemical Co., undated). Isoprene feedstock recovered from C₅ hydrocarbon refinery mixtures contains about 20% isoprene (Chevron Chemical Co., undated).

1.1.5 Analysis

The US Environmental Protection Agency has proposed methods for the analysis of volatile 'priority pollutants' in water [EPA Method 624] by gas chromatography-mass spectrometry and purge-and-trap techniques. Isoprene, at a detection limit of 3 µg/L, was one of the compounds studied (Spingarn *et al.*, 1982).

A method for the analysis of volatile organic compounds in air samples involves gas chromatography with flame ionization detection and coupled fused silica capillary columns of different polarity and length in order to separate the complex mixtures. Isoprene was detected by this method in atmospheric and alveolar air samples from exposed workers (Clair *et al.*, 1991).

A method for the collection and assay of volatile compounds, including isoprene, in breath involves drawing a sample through a water trap, then through an adsorptive trap of graphitized carbon. The sample is eluted by thermal desorption, concentrated by two-stage cryofocusing, then assayed by gas chromatography with flame ionization and flame photometric detection (Phillips & Greenberg, 1991).

An analytical method for the determination of non-methane hydrocarbons, including isoprene, in air makes use of gas chromatography and simultaneous programming of pressure and temperature on a capillary column. A combination of on-column cryofocusing and gas chromatographic reinjection was used, with a detection limit of 2 pg (Matuška *et al.*, 1986).

1.2 Production and use

1.2.1 Production

Isoprene was first isolated in 1860 during the pyrolysis of natural rubber. The reverse reaction, polymerization of isoprene to poly(*cis*-1,4-isoprene), which has a structure corresponding to that of natural rubber, was the subject of intensive effort. The first successful attempts were reported in 1954 and 1955 by the Goodrich Gulf (using an

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

aluminium–titanium Ziegler catalyst) and Firestone companies (using an alkyl lithium catalyst). Isoprene was commonly prepared on a laboratory scale by thermolysis of turpentine oil (the so-called isoprene lamp). Pyrolysis of dipentene (limonene) was used in the USA early in the Second World War as a commercial source of isoprene (Bibb process). Isoprene itself was of no commercial importance until after the Second World War, when cost-effective methods were developed for obtaining it from petrochemical sources (Weitz & Loser, 1989).

The first commercial synthesis of isoprene, the Goodyear Scientific Design isoprene synthesis, begins with dimerization of propylene to 2-methyl-1-pentene. This is then isomerized to 2-methyl-2-pentene, which is subsequently cracked with loss of methane to give isoprene. The most frequently used synthetic procedure is acid-catalysed addition of formaldehyde to isobutene (Prins reaction). Production facilities of this type are currently in operation in Japan and the former USSR (Weitz & Loser, 1989).

One-step dehydrogenation of isopentane to isoprene can be carried out according to the Houdry–Catadiene procedure (chromic oxide–aluminium oxide catalyst, about 600 °C, and about 7 kPa), with a yield of 52%. Isoprene is prepared commercially in this way in the former USSR. It has been produced by dehydrogenation of methylbutenes in the USA and the Netherlands (Weitz & Loser, 1989).

The primary source of isoprene today is as a by-product in the production of ethylene by naphtha cracking. A solvent extraction process very similar to that for 1,3-butadiene is used. The yield of isoprene is typically 2–5 wt% based on ethylene, although it may be increased by starting with a heavier raw material such as gas oil. With regard to energy consumption, recovery of isoprene from crack fractions is considerably more efficient than synthesizing the compound chemically. Increasingly heavier raw materials are being used in Europe and the USA for ethylene production in crackers, thereby increasing the quantity of isoprene by-product. The most appropriate solvents for production-scale separation of isoprene from other hydrocarbons include *N*-methylpyrrolidone, dimethylformamide and acetonitrile (Weitz & Loser, 1989; Bouton, 1992).

Information available in 1991 indicated that isoprene was produced by five companies in the USA, three in Japan and one each in the Netherlands and the United Kingdom (Chemical Information Services Ltd, 1991). Isoprene is produced in large quantities at several locations in the former USSR, which has a capacity of about 1000 thousand tonnes per year (Weitz & Loser, 1989). Production of isoprene in the USA was 230 thousand tonnes in 1981, 63 thousand tonnes in 1986 and 214 thousand tonnes in 1991 (US International Trade Commission, 1982, 1987, 1993). Production in Japan was 102 thousand tonnes in 1986 and 76 thousand tonnes in 1992. In western Europe, production in 1992 was approximately 15 thousand tonnes (Smith, 1993).

1.2.2 Use

Isoprene is used primarily for the synthesis of isoprene rubber, poly(*cis*-1,4-isoprene), which is used mostly for the production of vehicle tyres (Weitz & Loser, 1989). The second largest market for isoprene has been the manufacture of block polymers containing styrene (styrene–isoprene–styrene, or SIS, polymers), which are especially useful as thermoplastic rubbers and as pressure-sensitive or thermosetting adhesives. Smaller amounts of isoprene

are used in the production of butyl rubber (isobutene-isoprene copolymer). The distinctive features of butyl rubber include its low gas permeability, so that it is used in the construction of hoses and as a liner in tubeless tyres (Weitz & Loser, 1989).

The only chemical reaction of isoprene of commercial importance (other than polymerization) is its conversion to terpenes, e.g. citral, linalool, ionones, myrcene, L-menthol, *N,N*-diethylnerylamine, geraniol and nerolidols, which are used extensively in flavours and fragrances (Weitz & Loser, 1989).

1.3 Occurrence

Isoprene occurs widely in nature at low concentrations. It has been estimated that isoprene emissions from plants (associated with photosynthesis) amount to one-half of the total non-methane hydrocarbon emissions from the biosphere (Loreto & Sharkey, 1993). It is present in roasted coffee, in the gas phase of tobacco smoke (Graedel *et al.*, 1986; Löfroth *et al.*, 1989; Weitz & Loser, 1989), in gasoline and turbine automobile exhausts (Katzman & Libby, 1975; Hampton *et al.*, 1983; Stump & Dropkin, 1985; Graedel *et al.*, 1986), and can be regarded as a precursor of polycyclic aromatic compounds (Weitz & Loser, 1989). Isoprene is the basic structural unit of countless natural products, including natural rubber and the terpenes, and of biologically active important substances such as vitamins A and K (Saltman, 1981) and the steroid sex hormones. The biosynthesis of rubber and other natural products containing the isoprene skeleton proceeds not via isoprene itself, but rather via mevalonic acid (3,5-dihydroxy-3-methylpentanoic acid) (Stump & Dropkin, 1985).

1.3.1 Natural occurrence

Isoprene is the predominant hydrocarbon emitted by a number of deciduous forest species, including oak, poplar, sycamore and willow (Tingey, 1981; Shaw *et al.*, 1983; Lamb *et al.*, 1984; Isidorov *et al.*, 1985; MacKenzie *et al.*, 1991). It is also found in trace amounts in the emissions of coniferous trees and is a component of the volatile emissions of a number of shrubs, ferns, mosses and grasses (Altshuller, 1983; Winer *et al.*, 1989, 1992). Isoprene emission rates from foliage have been found to be in the range of 2.3–45 µg/g per h. Within forest canopies, the concentrations of isoprene and monoterpenes have been reported to range between [10 and 100 ppb carbon (ppbC)], while concentrations outside the canopy range from [1 to 10 ppbC] (Khalil & Rasmussen, 1992). The mean flux of isoprene from an isolated grove of oak trees was determined in two tracer studies to be 6780 and 8110 µg/m² per h (geometric means) (Lamb *et al.*, 1986).

Isoprene is produced endogenously in humans (see section 4.1).

1.3.2 Occupational exposure

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 3700 US employees were potentially exposed occupationally to isoprene (US National Institute for Occupational Safety and Health, 1993). Of this number, 93% were estimated to be exposed to isoprene and 7% to be exposed to materials containing isoprene. The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

Exposures to isoprene may occur during the manufacture of synthetic rubber and elastomers. Only two dated reports of occupational exposures to isoprene have been published. Pigolev (1968) reported area samples averaging 40 mg/m^3 in polymerization and rubber separation shops in Russia. The concentration exceeded 40 mg/m^3 in 48 of 140 air samples, with a maximal level of 52 mg/m^3 . In an earlier investigation in a Russian synthetic rubber plant, air concentrations of isoprene ranged from 17 to 28 mg/m^3 in a raw materials shop (mean, 19 mg/m^3), from 4 to 37 mg/m^3 in the polymerization department (mean, 22 mg/m^3) and from 3 to 18 mg/m^3 in the rubber separation department (mean, 8 mg/m^3) (Faustov, 1972).

1.3.3 Air

Isoprene enters the atmosphere as emissions from vegetation and emissions formed during wood pulping, biomass combustion and rubber abrasion (Graedel *et al.*, 1986). Emission rates and ambient concentrations measured in various field and laboratory studies have been reviewed (Shaw *et al.*, 1983; Graedel *et al.*, 1986; Lamb *et al.*, 1986, 1987).

The global annual emission of isoprene in 1988 was estimated to be 285 million tonnes (Turner *et al.*, 1991). Zimmerman *et al.* (1978) estimated an annual foliar isoprene emission of 350 million tonnes carbon [400 million tonnes isoprene], and Rasmussen and Khalil (1988) estimated a total annual isoprene emission of 450 million tonnes. The annual biogenic emission of isoprene in tropical Australia was estimated to be about 25 million tonnes carbon [28 million tonnes isoprene] (uncertainty range, 6–100 million tonnes carbon) (Ayers & Gillett, 1988). The biogenic isoprene emission rate for the United Kingdom was estimated to be 2000 tonnes per year for the years 1985 and 1989 (Hewitt & Street, 1992). The total rate of emission of isoprene from deciduous forests in the USA has been estimated to be 4.9 million tonnes per year (Lamb *et al.*, 1987), with the greatest emissions in the summer and with the southeast accounting for approximately 48% of the isoprene emitted (Altshuller, 1983). It has been estimated that 15 million tonnes per year of isoprene are emitted from the entire contiguous USA (Altshuller, 1983).

In studies conducted in US cities, the concentrations of isoprene in ambient air ranged from 0.005 to 90 ppbC [$0.003\text{--}50 \text{ }\mu\text{g/m}^3$] (Lonneman *et al.*, 1979; Arnts & Meeks, 1981; Winer *et al.*, 1981; Khalil & Rasmussen, 1992; US National Library of Medicine, 1993a). Natural isoprene concentrations in Japan during average wet and clear seasons were 0.56 and 1.9 ppbC [0.31 and $1.1 \text{ }\mu\text{g/m}^3$], respectively. The atmospheric concentrations of isoprene at Auch, France, were 0.088–0.675 ppb by volume (ppbv) [$0.25\text{--}1.9 \text{ }\mu\text{g/m}^3$] at ground level, 0.017 ppbv [$0.05 \text{ }\mu\text{g/m}^3$] at 1160 m altitude and 0.160 ppbv [$0.45 \text{ }\mu\text{g/m}^3$] at 270 m; it was not detected at 2900 m (Kanakidou *et al.*, 1989).

Isoprene has been detected in emissions from wood stoves and fireplaces at concentrations ranging from 0.003 to 0.130 ppbC/ppm as CO_2 (Edgerton *et al.*, 1986). In another study, it was detected at a concentration of 7 ppb [$20.0 \text{ }\mu\text{g/m}^3$] in woodsmoke (Kleindienst *et al.*, 1986). It was detected at a concentration of 0.46 mg/m^3 in an atmospheric grab sample taken near an oil fire (Perry, 1975; US National Library of Medicine, 1993a) and was detected in five of 10 samples of exhaust from turbojet engines at concentrations ranging from 0.05 to 19.8 ppmC [$0.028\text{--}11 \text{ mg/m}^3$] (Katzman & Libby, 1975). The average airborne yield of isoprene from cigarette smoke has been estimated to be 3.1 mg/cigarette. Isoprene

levels of 85 and 150 $\mu\text{g}/\text{m}^3$ were determined in two studies of tavern air during normal smoking conditions; the corresponding outdoor air concentrations at the time were 2 and $< 1 \mu\text{g}/\text{m}^3$ isoprene (Löfroth *et al.*, 1989).

1.3.4 Other

In a pilot study of pollutants in the breast milk of women living in four urban industrial areas in the USA, isoprene was detected in one of eight samples (Pellizzari *et al.*, 1982). In a pilot study of volatile organic compounds in 224 samples of the exhaled breath of 28 non-smoking volunteers, isoprene was detected in about 50% of the samples at concentrations ranging from 0.2 to 0.6 ng/L (Krotoszynski *et al.*, 1977) (see also p. 221).

1.4 Regulations and guidelines

Most countries have not established standards or guidelines for occupational exposures to isoprene (American Conference of Governmental Industrial Hygienists, 1993; ILO, 1993; UNEP, 1993). The short-term exposure limit in Russia is 40 mg/m^3 , and the time-weighted average concentration is 100 mg/m^3 in Poland and 10 mg/m^3 in Bulgaria (ILO, 1993; UNEP, 1993; US National Library of Medicine, 1993b).

The US Food and Drug Administration (1993) permits use of isoprene in certain polymeric products in contact with food.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

3.1.1 Mouse

Groups of 40 male B6C3F1 mice, six to eight weeks old, were exposed to 0 (control), 70, 220, 700, 2200 or 7000 ppm [194–19 457 mg/m^3] isoprene (purity, $> 99\%$) vapours by inhalation (whole-body exposure) for 6 h per day on five days a week for six months. At the end of the exposure period, 10 mice per group were killed for gross and microscopic examination. The remaining 30 mice per group were maintained for a recovery period of six months without additional exposure to isoprene. Survival at termination of the study (12 months) was 27, 28, 28, 27, 26 and 21 for the control and exposed groups, respectively. Exposure to isoprene was associated with significant increases in the incidences of tumours at four sites, with the following incidences for control and treated groups: alveolar/bronchiolar adenoma or carcinoma: 2, 2, 1, 5, 10 and 9 ($p < 0.001$, logistic regression trend test); Harderian gland adenoma: 2, 6, 4, 14, 13 and 12 ($p < 0.001$, logistic regression trend test);

hepatocellular adenoma or carcinoma: 7, 3, 7, 15, 18 and 17 ($p < 0.001$, logistic regression trend test); forestomach squamous-cell papilloma or carcinoma: 0, 0, 0, 1, 4 and 6 ($p = 0.02$, incidental tumour test). Non-neoplastic lesions related to treatment included forestomach squamous-cell hyperplasia, lung alveolar hyperplasia, nasal olfactory degeneration and spinal cord degeneration (US National Toxicology Program, 1994).

3.1.2 Rat

Groups of 40 male Fischer 344/N rats, six to eight weeks old, were exposed to 0 (control), 70, 220, 700, 2200 or 7000 ppm [$194\text{--}19\,457\text{ mg/m}^3$] isoprene (purity, $> 99\%$) vapours by inhalation (whole-body exposure) for 6 h per day on five days a week for six months. At the end of the six-month exposure period, 10 rats per group were killed. The incidence of interstitial-cell hyperplasia of the testis was significantly increased in the 7000-ppm exposure group ($p < 0.01$) over that in the controls and other exposure groups (1/10, 1/10, 3/10, 1/10, 3/10 and 10/10). The remaining 30 rats per group were maintained for a recovery period of six months without additional exposure to isoprene. There was no treatment-related effect on body weight or survival. Survival at termination of the study (12 months) was 30, 30, 29, 30, 30 and 30 for the control and exposed groups, respectively. In comparison with the incidence in controls, there was a slight increase ($p = 0.02$, Cochran-Armitage trend test) in the incidence of interstitial-cell adenoma of the testis (3/30 controls *versus* 3/30, 4/30, 7/30, 8/30 and 9/30 treated rats). There was no significant treatment-related increase in any other type of tumour (US National Toxicology Program, 1994). [The Working Group noted the short duration of the study and that the design prevented adequate evaluation of carcinogenic potential. It was also noted that the spontaneous incidence of interstitial-cell tumours of the testis in this strain of rat is high at two years.]

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

Isoprene is formed endogenously, probably from mevalonic acid (Deneris *et al.*, 1984), a precursor of cholesterol biosynthesis. The endogenous production rate of isoprene was calculated to be $0.15\text{ }\mu\text{mol/kg per h}$ (Hartmann & Kessler, 1990). Concentrations in the blood range between 15 and 70 nmol/L (mean, 37 nmol/L [2.5 ng/ml]) (Cailleux *et al.*, 1992). Isoprene is also found in human breath at concentrations in the range of 10–30 nmol/L (Jansson & Larsson, 1969; DeMaster & Nagasawa, 1978; Cailleux & Allain, 1989). The quantity exhaled per day per individual was estimated to be 2–4 mg (Gelmont *et al.*, 1981).

4.1.2 Experimental systems

The rate of endogenous production of isoprene was determined to be $1.9\text{ }\mu\text{mol/kg per h}$ in rats and $1.9\text{ }\mu\text{mol/kg per h}$ in mice (Peter *et al.*, 1987).

1,2-Epoxy-2-methyl-3-butene was the major metabolite of isoprene formed by mouse liver microsomes; 3,4-epoxy-2-methyl-1-butene was a minor metabolite (20%) (Del Monte *et al.*, 1985; see Figure 1). 3,4-Epoxy-2-methyl-1-butene, but not 1,2-epoxy-2-methyl-3-butene, can be further metabolized to isoprene diepoxide. Microsomes from mice and Syrian hamsters showed a six-fold higher maximal metabolic velocity (V_{\max}) for the latter reaction than those from rats and rabbits: 1.6 *versus* 0.25 nmol diepoxide/mg protein per min. Pretreatment with phenobarbital increased the V_{\max} for this reaction by 3–20 times (Longo *et al.*, 1985).

The maximal metabolic elimination rates estimated from experiments in a closed inhalation system were 130 and 400 $\mu\text{mol/kg}$ per h in male Wistar rats and B6C3F1 mice, respectively. The half-lives of isoprene were 6.8 min in rats and 4.4 min in mice. At atmospheric concentrations above 300 ppm [837 mg/m^3], the rate of metabolism was no longer proportional to concentration (Peter *et al.*, 1987).

Male Fischer 344 rats exposed by nose-only inhalation for 6 h to 8, 260, 1480 and 8200 ppm [23, 738, 4200 and 23 268 mg/m^3] [$4\text{-}^{14}\text{C}$]isoprene retained 19, 9, 6 and 5% of the inhaled radioactivity, respectively. About 75% of the retained isoprene radioactivity was excreted in urine within 66 h. Liver, blood and, especially, fat were the tissues that contained most isoprene and metabolites. In the inhalation phase, respiratory tract tissues contained concentrations of volatile metabolites substantially out of proportion to their masses relative to liver and blood, which was interpreted to indicate metabolism in the respiratory tract. Most of the radioactivity in blood (> 85%) was associated with material of low volatility, probably mostly conjugates or tetrols. Between 0.031% (at 8 ppm) and 0.002% (at 8200 ppm) of the inhaled $4\text{-}^{14}\text{C}$ label was tentatively identified as isoprene diepoxide. Under the assumption that all radioactive material with the volatility of the diepoxide was indeed the diepoxide, blood diepoxide concentrations of 0.37, 7.4, 15 and 17 mmol/L were derived from 6-h exposures to 8, 260, 1480 and 8200 ppm, respectively (Dahl *et al.*, 1987). A species difference was demonstrated in a two-compartment model of isoprene pharmacokinetics. Both rats and mice exhibited saturation kinetics when exposed to isoprene at concentrations above 300 ppm; however, the V_{\max} in mice was determined to be 400 $\mu\text{mol/h}$ per kg, or more than three times that in rats (130 $\mu\text{mol/h}$ per kg), implying that mice are sensitive with regard to isoprene metabolism (Peter *et al.*, 1987).

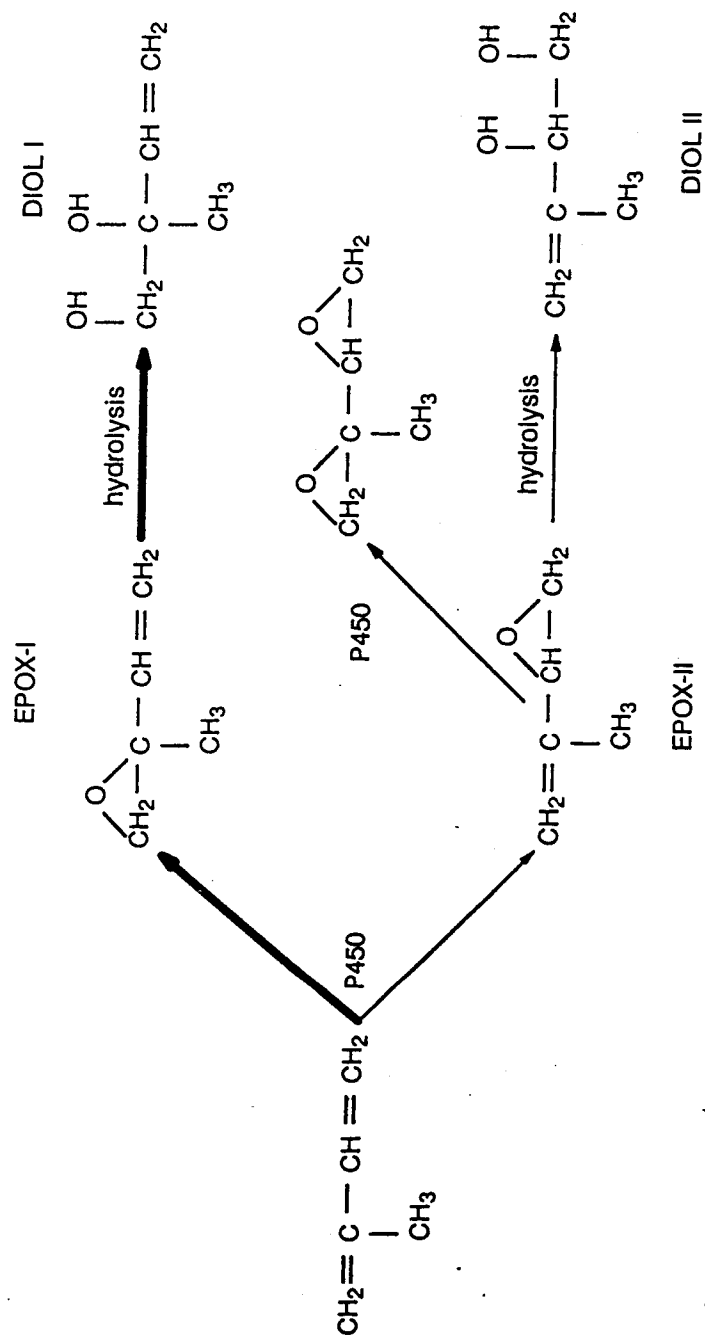
Male B6C3F1 mice exposed to 20, 200 and 2000 ppm [57, 568 and 5675 mg/m^3] ^{14}C -isoprene rapidly reached steady-state levels at which the blood concentrations of isoprene were 24.8, 830 and 6800 ng/ml, respectively (Bond *et al.*, 1991). Radioactivity in haemoglobin, measured 24 h after a single intraperitoneal injection into male Sprague-Dawley rats and male B6C3F1 mice, was linearly related to the administered dose up to 500 $\mu\text{mol/kg}$ and showed the same slope for both species (Sun *et al.*, 1989).

4.2 Toxic effects

4.2.1 Humans

Catarrhal inflammation, subtrochic and atrophic processes in the upper respiratory tract, and deterioration of olfaction were noted in isoprene rubber production workers.

Fig. 1. Microsomal metabolic pathways of isoprene



From Gervasi & Longo (1990)

EPOX-I, 1,2-epoxy-2-methyl-3-butene; EPOX-II, 3,4-epoxy-2-methyl-1-butene; P450, cytochrome P450

Major metabolic pathways are indicated by thick arrows and minor pathways by thin arrows.

Prevalence and degree were correlated with increasing length of service (Mitin, 1969; Sandmeyer, 1981).

4.2.2 *Experimental systems*

Exposure of Fischer 344 rats of each sex to 0, 438, 875, 1750, 3500 or 7000 ppm [1222–19 530 mg/m³] isoprene vapours by inhalation for 6 h a day on five days a week for two weeks had no effect on survival, body weight gain, clinical signs, haematological parameters or clinical chemical measurements and did not produce gross or microscopic lesions. Male B6C3F1 mice, however, had reduced body weight gain, atrophy of the thymus and testis (at 7000 ppm only), olfactory epithelial degeneration (at ≥ 1750 ppm), vacuolized liver cytoplasm and forestomach epithelial hyperplasia (at ≥ 438 ppm). The last effect was also seen in female mice. In both male and female mice in all exposure groups, there were reductions in erythrocyte numbers, haemoglobin concentrations and volume of packed erythrocytes; the numbers of reticulocytes and polychromatic erythrocytes were not increased (Melnick *et al.*, 1990).

Exposure of male Fischer 344 rats and male B6C3F1 mice to 0, 70, 220, 700, 2200 or 7000 ppm [195–19 530 mg/m³] isoprene vapours by inhalation for 6 h a day on five days a week for 26 weeks induced exposure-related neoplastic and proliferative lesions in the lung, Harderian gland and forestomach in mice. Interstitial-cell hyperplasia of the testis was induced in rats exposed to 7000 ppm isoprene (US National Toxicology Program, 1994).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The developmental toxicity of isoprene was reported in abstracts by Mast *et al.* (1989, 1990). Swiss CD-1 mice and Sprague-Dawley rats were exposed to 0, 280, 1400 or 7000 ppm [781–19 530 mg/m³] isoprene vapour for 6 h per day on seven days per week on days 6–17 (mice) or days 6–19 of gestation (rats). In mice, exposure to 7000 ppm isoprene reduced maternal weight gain, and exposure to any dose reduced fetal body weight. An increase in the incidence of supernumerary ribs was observed at 7000 ppm, but there was no increase in fetal malformations. In rats, there was no adverse effect on the dams or on any reproductive index at any dose level, and there was no increase in the incidence of either fetal malformations or variations, other than reduced ossification of the vertebral centra at 7000 ppm.

4.4 Genetic and related effects (see also Table 1 and Appendices 1 and 2)

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Isoprene did not induce mutation in *Salmonella typhimurium*.

Exposure of male B6C3F1 mice to isoprene by inhalation produced increases in the frequencies of micronuclei in circulating erythrocytes and of sister chromatid exchange in bone-marrow cells, but no chromosomal aberrations were seen.

Neither the major metabolite in mouse liver microsomes, 1,2-epoxy-2-methyl-3-butene, nor an important minor metabolite, 3,4-epoxy-2-methyl-1-butene, was mutagenic to *S. typhimurium* TA98 or TA100. The latter metabolite can be metabolized further to 2-methyl-1,2:3,4-diepoxybutane, which was mutagenic to both strains. This compound may be responsible for the activity of isoprene *in vivo*; it is an analogue of 1,2:3,4-diepoxybutane, which can induce chromosomal aberrations and sister chromatid exchange in mouse bone marrow (Conner *et al.*, 1983; Walk *et al.*, 1987).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Isoprene is produced by dehydrogenation of isopentane or as a by-product in the production of ethylene via naphtha cracking. It is used mainly in the production of isoprene rubber (used in vehicle tyres), block polymers containing styrene (used as thermoplastic rubbers) and pressure-sensitive adhesives and in butyl rubber used for construction of hoses and tyres. Large quantities of isoprene are produced and released into the atmosphere by vegetation. Few data are available on occupational exposure to isoprene.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Isoprene was tested for carcinogenicity in male mice and male rats exposed by inhalation in one-year studies. In mice, exposure to isoprene resulted in increased incidences of benign and malignant tumours of the lung, liver and forestomach and of Harderian gland adenomas. The study by inhalation in rats was inadequate for an assessment of carcinogenicity.

5.4 Other relevant data

Isoprene is formed endogenously and is present in exhaled air from man and rodents. Isoprene concentrations in human blood, from apparently endogenous sources, are approximately one-tenth of the concentrations reached in mouse blood following exposure to 20 ppm [56 mg/m³] in the atmosphere. At low concentrations, less inhaled isoprene is retained in mice than in rats. Haemoglobin adducts of isoprene can be formed in both mice and rats, but at equal retained doses mice form twice as much haemoglobin adduct as rats. Toxic responses have been observed only in mice. Isoprene can be oxidized sequentially by microsomal enzymes to 2-methyl-1,2:3,4-diepoxybutane.

Table 1. Genetic and related effects of isoprene

Test system	Result ^a	Dose ^b (LED/HID)		Reference
		Without exogenous metabolic system	With exogenous metabolic system	
Isoprene				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	5000.0000	Mortelmans <i>et al.</i> (1986)
SA2, <i>Salmonella typhimurium</i> TA102, reverse mutation	-	0	0.0000	Kushi <i>et al.</i> (1985) (abstr.)
SA3, <i>Salmonella typhimurium</i> TA1530, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA4, <i>Salmonella typhimurium</i> TA104, reverse mutation	-	0	0.0000	Kushi <i>et al.</i> (1985) (abstr.)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	5000.0000	Mortelmans <i>et al.</i> (1986)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	5000.0000	Mortelmans <i>et al.</i> (1986)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	5000.0000	Mortelmans <i>et al.</i> (1986)
SVA, Sister chromatid exchange, mouse bone-marrow cells <i>in vivo</i>	+	+	430.0000, inhal. 6 h × 12	Tice <i>et al.</i> (1988)
MVM, Micronucleus test, mouse peripheral red blood cells <i>in vivo</i>	+	+	430.0000, inhal. 6 h × 12	Tice <i>et al.</i> (1988)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	-	-	6900.0000, inhal. 6 h × 12	Tice <i>et al.</i> (1988)
Protein binding				
BVP, Binding (covalent) to mouse haemoglobin <i>in vivo</i>	+	+	2.0000 × 1 ip	Sun <i>et al.</i> (1989)
BVP, Binding (covalent) to mouse haemoglobin <i>in vivo</i>	+	+	60.0000, inhal. 6 h	Bond <i>et al.</i> (1991)
BVP, Binding (covalent) to rat haemoglobin <i>in vivo</i>	+	+	2.0000 × 1 ip	Sun <i>et al.</i> (1989)
1,2-Epoxy-2-methylbutane				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	0	2600.0000	Gervasi <i>et al.</i> (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	2600.0000	Gervasi <i>et al.</i> (1985)

Table 1 (contd)

Test system	Result ^a	Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system	
3,4-Epoxy-2-methyl-1-butene			
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	0	Gervasi <i>et al.</i> (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	Gervasi <i>et al.</i> (1985)
2-Methyl-1,2,3,4-diepoxybutane			
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	Gervasi <i>et al.</i> (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	+	0	Gervasi <i>et al.</i> (1985)

^a +, positive; -, negative; 0, not tested^b In-vitro tests, µg/ml; in-vivo tests, mg/kg bw^c Atmospheric concentration

Exposure of mice and rats to isoprene by inhalation had no adverse effect on reproduction.

No data were available on the genetic and related effects of isoprene in humans. Isoprene induced micronuclei in mouse circulating erythrocytes and sister chromatid exchange but not chromosomal aberrations in mouse bone marrow *in vivo*.

Neither isoprene nor its primary metabolites, 3,4-epoxy-2-methyl-1-butene and 1,2-epoxy-2-methyl-3-butene, were mutagenic to bacteria. 2-Methyl-1,2:3,4-diepoxybutane, a metabolite of 3,4-epoxy-2-methyl-1-butene, was mutagenic to *Salmonella typhimurium*.

5.5 Evaluation¹

There is *inadequate evidence* in humans for the carcinogenicity of isoprene.

There is *sufficient evidence* in experimental animals for the carcinogenicity of isoprene.

Overall evaluation

Isoprene is *possibly carcinogenic to humans (Group 2B)*.

6. References

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¹For definition of the italicized terms, see Preamble, pp. 27-30.

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APPENDIX B

Report on Carcinogens (RoC), 9th Edition Review Summary

**Report on Carcinogens (RoC), 9th Edition
Review Summary**

Isoprene

NOMINATION

Review based on results of an NTP Bioassay of Isoprene (1995, 1997) reporting clear evidence of benign and malignant tumor formation at multiple organ sites in multiple species of experimental animals.

DISCUSSION

Isoprene is used in the production of 95% polyisoprene and 2% isoprene-butadiene copolymers and is also the monomeric unit of natural rubber and naturally occurring terpenes and steroids. There is clear evidence of benign and malignant tumor formation at multiple organ sites from inhalation studies of isoprene in multiple species of experimental animals. Isoprene has a close structural relationship to 1,3-butadiene. The recommendations from the three NTP reviews of this nomination are as follows:

Review Committee	Recommendation	Vote
NIEHS (RG1)	list as a reasonably anticipated human carcinogen	6 yes/0 no
NTP EC Working Group (RG2)	list as a reasonably anticipated human carcinogen	6 yes/0 no/1 a*
NTP Board RoC Subcommittee	list as a reasonably anticipated human carcinogen	13 yes/0 no

*a-abstentions

Public Comments Received

A total of 2 public comments were received, both against listing in the RoC in any category.